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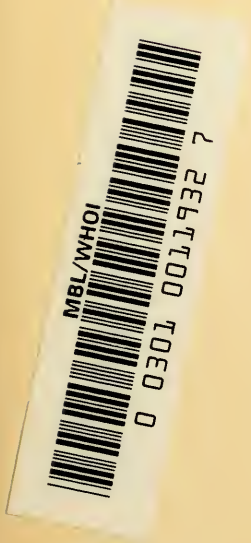

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REGENERATION

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REGENERATION

REGENERATION

FROM A PHYSICO-CHEMICAL VIEWPOINT

BY
JACQUES LOEB
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First Edition

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PREFACE

About a generation ago, biologists devoted considerable time to a discussion of the vitalistic and mechanistic conception of life processes. The impulse for this discussion was given at that time by the experiments of Roux and of Driesch upon the development of eggs, parts of which had been destroyed or removed in the first stages of segmentation. It was generally or frequently observed that the development of the mutilated egg resulted in the formation of a normal organism. Driesch maintained that this phenomenon could not be adequately explained on a purely physico-chemical basis, but that in addition a metaphysical guiding principle inherent in the organism as a whole was to be postulated. The opposite view was held by Roux. The controversy was never settled, for the simple reason that on account of the microscopic size of the egg cells the experiments of both authors had to be purely qualitative. An adequate explanation of natural phenomena is possible only on the basis of quantitative experiments and such an explanation consists in the derivation of the results from a rationalistic mathematical formula (a so-called "law") without the introduction of arbitrary constants.

Phenomena of regeneration occur not only in eggs but in adult animals and plants as well. The problem is, first, why does mutilation of an organism give rise to phenomena of growth which do not occur without mutilation, and second, why does the new growth frequently (though not always) result in some kind of restoration of the old form of the mutilated organism. The same mysterious "guiding principle," which had been suggested by the vitalists for the egg, was suggested also for the explanation of the seemingly purposeful character of regeneration in adult animals and plants, since regeneration leads frequently to the restoration of that form which is characteristic of the organism as a whole.

An enormous literature exists on the regeneration in both animals and plants, but the experiments reported were generally merely qualitative in character, and where an attempt at

measurements was made, the measurements led to no rationalistic correlation between the process of regeneration and the mutilation of the organism, for the reason that there was no clear conception concerning the quantity which it was necessary to measure.[1] This is only possible if we have a clear idea of the physico-chemical relation which it is intended or necessary to test.

The writer has for a number of years conducted quantitative experiments on the regeneration of a plant, *Bryophyllum calycinum*, which have made it possible to correlate the process of regeneration with the quantity of chemical material. By comparing the dry weight of the regenerated shoots and roots with the dry weight of the leaves or stems which were used for regeneration, it could be shown that in the presence of light the quantity of regeneration was under equal conditions of illumination, temperature, and in equal time in direct proportion to the mass of the leaves or stems from which regeneration started. If we make the legitimate assumption that the material required for the formation of new shoots or roots was under the conditions of our experiments produced by the chlorophyll contained in the leaf or stem, it follows that the quantity of regeneration is determined in this case by the mass of material available in the stem or leaf for synthetic processes. Under the guidance of this mass relation (which may be, in part at least, identical with the law of mass action), it could be shown that mutilation of the plant leads to a collection of sap in places where it would not have collected without the mutilation. This accounts for the fact that mutilation leads to growth in places of the organism where no growth would have occurred without mutilation. The process of regeneration was thus revealed as a purely physico-chemical

[1] In addition to the literature mentioned in the text, the reader is referred to Barfurth, D., *Methoden zur Erforschung der Regeneration bei Tieren*, in Abderhalden's *Handb. biol. Arbeitsmeth.*, Abt. V, Teil 3, Heft 1, 1921, and Barfurth's and Driesch's reviews of the literature in *Ergeb. Anatom. u. Entwicklungsgesch.*, Vol. xxii, 1914, and earlier volumes. Also Korschelt, E., *Regeneration und Transplantation*, Jena, 1907; Przibram, H., *Experimental-Zoologie*, ii, *Regeneration*, Leipzig and Wien, 1909. The most interesting recent publications on regeneration are from Przibram's laboratory, by himself and his collaborators. For the problem of growth which is fundamental for regeneration, the reader is referred to Robertson, T. B., "The Chemical Basis of Growth and Senescence, Philadelphia and London, 1923.

phenomenon, leaving no necessity nor room for the postulation of a guiding principle aside from the purely physico-chemical forces.

The writer has confined himself to experiments on one form only, which, however, is unusually favorable for quantitative work on regeneration. The reason for this restriction lay in the fact that it was his intention to find a rationalistic law which could be used as a guide in further experiments on regeneration. For this purpose it was essential to confine himself in the beginning to the elaboration of this law in an organism which permitted the carrying out of the necessary quantitative experiments, and *Bryophyllum* met this prerequisite. We are already in possession of an enormous number of enigmatic though often interesting observations on regeneration in different animals and plants, and it seemed of little value to add to this store of riddles. It is primarily not more facts which are needed in this field but a method and a principle which allow us to pass from the stage of blind empiricism to the stage of an oriented search. As long as the investigation of a natural phenomenon is in the stage of blind empiricism we never know what to look for in our experiments nor what to measure, and we are not able to judge whether we are on the road to progress or whether we are losing ourselves in a jungle of futile experiments. With a rationalistic law and a rationalistic method as guides, this danger is avoided. The writer believes that the mass relation referred to can render this service in the field of regeneration, and that the extremely simple method of correlating the dry weight of regenerated organs with the dry weight of the regenerating plant serves as a sufficiently safe method for a first approximation to exact results and conclusions.

The contents of the book is divided into two parts, the first correlating mutilation and regeneration on the basis of the mass relation, and the second making an attempt at a similar treatment of the problem of the polar character of regeneration.

The book is based on a number of papers published in the *Journal of General Physiology*, but it seemed advisable to put the main results into the form of a short monograph where the facts could be arranged in a more logical sequence than was possible in the original publications. The main experiments required for the proof of the theory were once more repeated before the book was written and in many cases these experiments were

incorporated in the book. In order to make it easier for the reader to visualize the experiments a large number of drawings from nature are introduced in the text, for which the writer is indebted to Miss M. Hedge of the Illustration Division of the Institute.

Jacques Loeb.

The Rockefeller Institute
of Medical Research,
New York.
January, 1924.

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PART I
MUTILATION AND REGENERATION

CHAPTER I

INTRODUCTION

1. Living organisms, as well as crystals, are characterized by a definite shape which is primarily determined by the chemical nature of their material. Both types of forms grow, but the mechanism of growth is different in the two types. Crystals grow in supersaturated, undercooled solutions of the molecules of which they are composed. Living cells grow in solutions of low concentrations of simpler compounds than those of which their cells are composed, and the growth of living cells is preceded by a synthesis of the large insoluble molecules of the cytoplasm and of the nucleus from the comparatively small molecules, such as amino-acids, sugars, etc.

It is very interesting that crystals and living organisms have also the phenomenon of regeneration in common. When a mutilated crystal is put into a supersaturated, undercooled solution of its substance, it restores its old form, and the same phenomenon of repair or restoration of the old form occurs in mutilated living organisms. The mechanism of regeneration is, however, also entirely different in the two cases, for the reason that the mechanism of growth (which gives rise to regeneration) is different.

Let *abcd* (Fig. 1) be the side of a cubical crystal such as NaCl. In a supersaturated, undercooled solution of NaCl, the crystal will grow slowly, always retaining its cubical form because the rate of growth is exactly equal at each element of the surface. This rate of growth is a relative minimum at the normal surfaces of a crystal. When now the crystal is mutilated, *e.g.*, when the angle at a is cut off so that the form a_1a_2-$bdca_1$ results (Fig. 2), the old normal surfaces, a_2b, bd, dc, and ca_1 continue to grow at the old minimal rate. The rate of the growth of the new surface a_1a_2 is, however, greater than that of the old normal surfaces, and this difference in the rate of growth depends on the angle of the new surface with the old surface where the growth is a minimum. As a consequence of this greater rate of growth of the new surface a_1a_2, this surface is bound to eliminate

itself in time for the following reason. Let us consider the point a_1 and one minute element of surface next to it on each of the two sides, a_1a_2 and a_1c. The minute elements of area adjacent to point a_1 on the side a_1c will continue to grow slowly, but the element of area adjacent to point a_1 on the surface a_1a_2 will grow more rapidly. As soon as this latter element reaches the level of a_1c, it will, on account of the change in orientation, grow with the minimal velocity characteristic of the old surface a_1c. The same will happen at the angle a_2, so that the area a_1a_2 is being steadily diminished. This is indicated diagrammatically in Fig. 3, where the inner polygon a_1a_2bdc is the side of the mutilated crystal at the beginning of the experiment, and the middle polygon $a_3a_4b_1d_1c_1$ is the side some time later. The rate

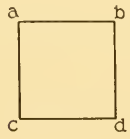

Fig. 1.

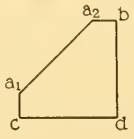

Fig. 2.

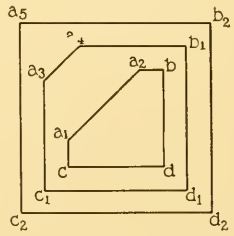

Fig. 3.

Figs. 1 to 3.—Diagrammatic representation of regeneration of mutilated cubical crystal. Figure 1, size of the crystal before mutilation. Figure 2, mutilated crystal (angle a replaced by area a_1a_2). Figure 3, elimination of the new area a_1a_2 by more rapid growth of this area.

of growth is indicated by the distance of the new lines from the old. The distance of a_3a_4 from a_1a_2 is greater than that of a_3c_1 from a_1c, etc. As a consequence a_3a_4 is smaller than a_1a_2 or, in other words, the wound of the crystal is "healing." This continues until the whole area a_1a_2 created by mutilation has disappeared again and the angle a_5 is reached. In this case the crystal has its old cubical form represented by the side $a_5b_2d_2c_2$. Hence the regeneration of the crystal is due to the fact that the area a_1a_2 (Figs. 2 and 3) created by mutilation is bound to eliminate itself by the more rapid growth of its elements, caused by the change in the orientation of the new surface through the mutilation.[1]

2. We will now pass in review briefly a few typical cases of regeneration in living organisms. It is at once much more

[1] Masing, G.: *Die Naturwissenschaften*, p. 895, 1922.

complicated and entirely different from that in crystals. Let us select as the simplest type of regeneration in living organisms that of a sea anemone, *Cerianthus membranaceus*. This organism may be described as a hollow cylindrical tube (Fig. 4), closed at one end, the aboral, and open at the opposite, the oral end. This latter end is surrounded by a row of tentacles. If we now cut out a square piece, *abcd* (Fig. 4), from the wall of the cylindrical tube, and keep it in sea water, the piece will live although it is no longer capable of taking up food. In due time, which, if the temperature is low, may be weeks or months, at one of the

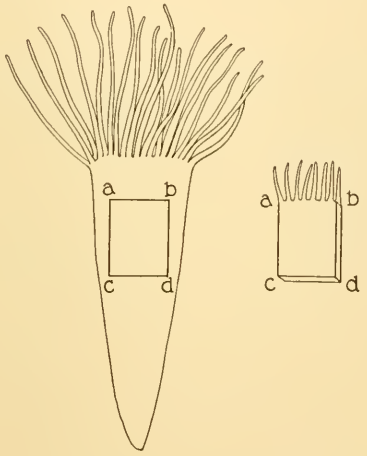

Fig. 4. Fig. 5.

Figs. 4 and 5.—Figure 4, diagrammatic view of an actinian from the wall of which a square piece, *abcd*, is cut out. Figure 5, this piece forms tentacles only on the side *ab*, which was originally oriented towards the oral pole.

four sides of the piece, namely, *ab* (Fig. 5), which was oriented towards the oral end of the animal, new tentacles will grow out, but no growth of this kind will appear on the other three sides of the piece. We may say that each isolated longitudinal element of the body wall of *Cerianthus* possesses the power of growing into a tentacle at its oral end, but that no appreciable growth in length occurs on any of the other three sides. This is an example of the so-called polar type of regeneration, which has occupied the attention of the biologists.[1]

[1] Loeb, J.: *Untersuchungen zur physiologischen Morphologie der Tiere*, II, Würzburg, 1891.

When merely a lateral incision is made through the body wall of *Cerianthus* (*abc*, in Fig. 6), tentacles will grow out from the lower edge *bc* of the cut. No growth of tentacles will occur from the other end, *ab*, and only the ordinary process of wound healing will take place here as a consequence of which the incision will be closed. The body of the animal will look after some time as in Fig. 7.

The sea water does not contain the material for the formation of the new tentacles. The tentacles are formed from material contained in the tissue sap which surrounds the cells and soluble material in this sap is synthesized by an action inside the cells

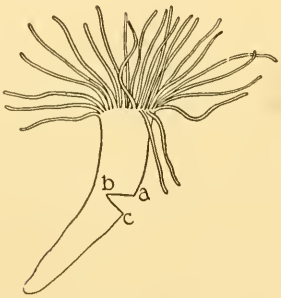

FIG. 6.

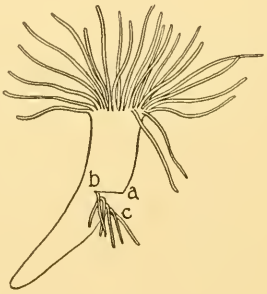

FIG. 7.

FIGS. 6 and 7.—Effect of lateral incision into the body of the actinian. Only the lower edge *bc* of the incision *abc* forms tentacles.

into the solid material forming the regenerated tentacles. Since the chromosomes of the nucleus determine the hereditary form their chemical constitution must in some way be responsible for the formation of tentacles in the regenerating piece.

As a second type may be selected the regeneration of fresh water planarians studied by Morgan.[1] When a piece, *acdb* (Fig. 8), is cut out from a planarian at right angles with the longitudinal axis of the animal the piece will regenerate a new head in front of *ac*, a new tail at the other end, *db*, and an organism as represented in Fig. 9 will result. When, however, the piece *acdb* is cut out obliquely from the body (Fig. 10) a tiny new head will be formed only in the foremost corner *a*, and a tiny new tail in the hindmost end *b* (Fig. 11). This type of regeneration differs typically from that of *Cerianthus* in that the regeneration is no longer the same for each longitudinal element but depends upon

[1] MORGAN, T. H.: "Regeneration," New York, 1901.

the angle at which the cut is made. In the case of *Cerianthus* each longitudinal element of the body wall will produce a tentacle at its oral end, no matter whether the cut is at right angles to the longitudinal axis of the animal or is made obliquely.

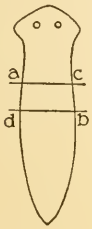

Fig. 8.

Fig. 9.

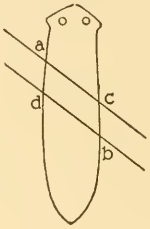

Fig. 10.

Fig. 11.

Figs. 8 and 9.—Figure 8, diagram of a fresh water planarian from which a piece, *acbd*, is cut. Figure 9, this piece forms a new head at its oral end *ac*, and a new tail at its basal end.

Figs. 10 and 11.—Regeneration of a piece, *acbd*, cut out obliquely from the planarian. A tiny new head is formed near the most apical corner *a* of the piece *acbd*, and a new tail at the most basal corner *b*. (*After Morgan.*)

As a third type we may select a hydroid *Tubularia* (Fig. 12), because it introduces a principle of regeneration existing only in living organisms, but not—as far as the writer is aware—in

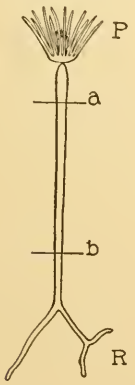

Fig. 12.

Fig. 13.

Figs. 12 and 13.—Figure 12, diagram of a normal hydroid, *Tubularia*, from which a piece, *ab*, is cut out. *P*, polyp; *R*, foot of the hydroid. Figure 13, heteromorphic regeneration of the piece *ab*, which forms a new polyp at either end.

crystals. *Tubularia* is a sessile marine organism with a long slender body, one end of which—which we will call the foot, *R* (Fig. 12)—is attached to solid bodies (rocks or pieces of wood),

while the other end has a polyp (or head), *P* (Fig. 12). The writer found that when a piece, *ab*, is cut out from the stem of a Tubularian and suspended in sea water, a new polyp, *d* and *c* (Fig. 13), is generally formed at either end of the body. In this case the regeneration of the mutilated piece does not result in the restoration of the old organism but in the production of an organism never found under natural conditions, since it terminates in a head at either end instead of terminating in a head at the oral and a foot at the aboral end. The substitution of one type of organ by another has been termed heteromorphosis[1] and a number of cases of heteromorphosis in regeneration have since been described.

A still different type of regeneration occurs in plants where shoots originate, as a rule, from preformed definite anlagen. Since this type of regeneration is the subject of this little volume, it need not be discussed in this introduction.[2]

No scientific explanation of these or other cases of regeneration in living organisms has been offered, if by a scientific explanation is meant a rationalistic mathematical theory based on quantitative measurements. The explanations offered were either purely verbalistic, such as the assumption of a guiding spirit in each organism which directs its growth according to a preconceived plan (the entelechy of Driesch, the morphaestesia of Noll, etc.), or they were based on assumptions that are plainly in contradiction with the facts. One assumption often repeated is that the wound produces specific "wound hormones." It can be stated at the outset that the idea of "wound stimuli" or "wound hormones" is excluded in the experiments on *Bryophyllum* with which this volume is concerned. When a leaf of *Bryophyllum* is removed from a plant and submersed with the apex in water (Fig. 14), those notches of the leaf which dip into the water give rise to a new shoot and to new roots. The only wound in this case is at the base of the petiole of the leaf, no regeneration occurring at or near the wound. The growth occurs only in the notches of the apex which in this case is farthest away from the wound. The growth will occur even if a small piece of stem is attached to

[1] Loeb, J.: *Untersuchungen zur physiologischen Morphologie der Tiere. I. Heteromorphose*, Würzburg, 1890.

[2] A collection of observations on regeneration in plants is found in Goebel, K., *Einleitung in die experimentelle Morphologie der Pflanzen*, Leipzig and Berlin, 1908.

the leaf so that there is no wound at the base of the petiole. It may even happen (though very rarely) that without any mutilation a leaf attached to a stem will give rise to tiny shoots. This was observed in the case of old plants in which probably the flow of sap from the leaf to the stem was interrupted. In these cases it is impossible to say that a "wound hormone" or a "wound stimulus" could have been responsible for the new growth and regeneration.

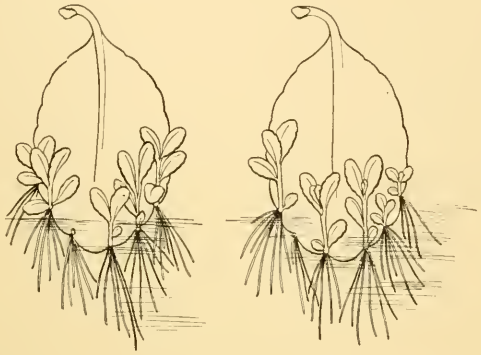

FIG. 14.—Isolated sister leaves of *Bryophyllum calycinum* dipping with their apices into water. Mar. 20 to Apr. 11, 1923. Equal masses of leaves producing equal masses of shoots and roots. Root and shoot formation only in wetted parts of the leaves.

It will be shown in this book that the only three assumptions necessary to explain the phenomenon of regeneration in *Bryophyllum calycinum* are, first, that under the influence of light the chlorophyll in the leaves or stems causes directly or indirectly the formation of those soluble substances which are required for growth, such as sugars, amino-acids, and other specific and non-specific substances which may be needed. The special nature of these substances does not concern us here beyond assuming that the leaf or the stem is able to produce all the substances required for the growth of new shoots or roots. The second assumption is that the mass of material, formed by the influence of light on chlorophyll, increases (under equal conditions of illumination, temperature, moisture, and solutes or gases in the environment) in proportion to the mass of chlorophyll. These two assumptions will not be seriously questioned. The third assumption is that the mass of chlorophyll of the leaf or stem used for regeneration remains fairly constant during the limited time the experiment

lasts. This is not strictly correct, since the new shoots formed also contain chlorophyll. But the experiment is terminated before this can cause an appreciable error. It can be shown that the dry weight of shoots or roots regenerated by a leaf or stem is (under equal conditions and in equal time) in direct proportion to the mass of leaves or stems in which regeneration occurs; and this simple relation will be designated briefly as the mass relation.

The reader will notice that the weight of shoots and roots produced by a leaf or stem is used as a measure of the rate of growth of the shoots and roots. The proportionality between mass of leaves or stems and the mass of shoots and roots produced by them no longer need to hold as soon as the growth of shoots and roots is no longer exclusively or predominantly a function of the assimilating activity of the leaf or stem.

Whether or not the mass relation is identical with the law of mass action may remain undecided as far as the contents of this book are concerned. The mass relation may certainly be at first the expression of the mass law, since the first anlagen of shoots and roots are equal regardless of the mass of the leaf or stem where the regeneration occurs. The mass of material diffusing into each anlage will increase with the mass of the leaf or stem (provided the light is not excluded), and the concentration of the solutes of the sap in each anlage will as a consequence increase in proportion with the mass of the leaf or stem in which the regeneration occurs. It is quite possible that the growing points of the young shoot will remain small and not vary materially in size during the first growth, and in this case the mass relation will remain identical with the mass law. But if the internodes of the new regenerated shoot grow in thickness the mass of growing cells in a large regenerated shoot will be greater than that in a smaller shoot, and in this case the mass relation may no longer be identical with the mass law. For this reason we shall only speak of the mass relation and not of the mass law. The mass relation, that is, the fact that the mass of shoots and roots regenerated varies in proportion with the mass of the leaf or stem where the regeneration occurs, is sufficiently simple and rationalistic to serve as a guide in the maze of the phenomena of regeneration.

CHAPTER II

GENERAL REMARKS ABOUT THE MATERIAL AND THE EXPERIMENTS

The organism used for the experiments was the plant *Bryophyllum calycinum*, which was raised in numerous specimens from a few leaves of this plant which the writer received nine years ago from Bermuda. When a leaf of this plant is detached from a stem and put on moist soil (or suspended in moist air) each one of its notches may give rise to roots and shoots from which new plants develop (Fig. 14). It has been stated that this is the usual way by which the plant propagates. The plant consists of a straight unbranched stem bearing 2 leaves in each node which grow and reach a large size. In the greenhouse the young leaves are thin in summer and become fleshy in winter. The plant sheds only the older leaves, *i.e.*, those nearest the base, while new leaves are continually formed at the apex. As a consequence the plants as they exist in the greenhouse have a straight unbranched vertical stem which may be as thick as a thumb and which may reach the height of 2 meters or more, with leaves in the upper nodes near the apex, while the lower nodes are free from leaves. Each node has, as stated, 2 leaves and the axis connecting the 2 leaves in one node is always at right angles to the axis connecting the 2 leaves in each of the 2 adjacent nodes. Only a small number of plants in the greenhouse produced flowers and these plants were exposed to the strongest light; the majority, kept in parts of the greenhouse where the illumination was not so good, never produced flowers. The plants used for the experiments belonged to this latter class.

The care these plants require is of special importance. In the greenhouse they are liable to suffer from insects, especially plant lice, from which it is necessary to keep them free. A second factor of importance is that these plants did not thrive in the rooms of the laboratory where illuminating gas was used or where people smoked. No quantitative experiments on regeneration could be made with any degree of satisfaction in the laboratory

rooms of the Institute, on account of the sensitiveness of these plants to certain contaminations of the air. All the experiments were carried out in a greenhouse in which the temperature varied between 20 and 30°C., and where it was never below 20°C. In the greenhouse no illuminating gas was used and no smoking was permitted.

Whoever wishes to repeat the writer's experiments must realize that the prerequisites of pure air, proper temperature, good illumination, and the protection of the plant against parasites must be fulfilled.

When leaves of *Bryophyllum calycinum* are separated from the stem each notch of the leaf may give rise to roots and a little later to shoots (Fig. 14). Such growth occurs only in the notches of the leaf. When the notches are cut out from an isolated leaf no root or shoot formation occurs in the rest of the leaf. As long as the leaf is part of a normal plant, these dormant buds in the notches will not, as a rule, grow out. Only in old plants and old leaves (where there is a suspicion that the flow of sap from leaf to stem is more or less completely blocked) may it happen that a leaf gives rise to roots and shoots while still connected with the stem.

The experiments were made in large aquaria which were filled to the height of 10 centimeters with water. To strings stretched across the top of the aquarium thin horizontal rods of iron were hooked, and from these the leaves or stems used for regeneration were suspended. To keep the air in the aquarium fairly moist, the top of the aquarium was covered loosely with a glass plate.

Bryophyllum has, as stated, 2 leaves in each node, which are of equal age and size, and which may be considered to possess approximately equal masses of chlorophyll and other substances per gram dry weight of leaf. Furthermore, the relative efficiency of the chlorophyll in the unit of mass of two sister leaves may be considered to be about the same. Hence the roots and shoots produced by equal masses of two sister leaves (detached from the plant) in equal times, under equal illumination and equal temperature, may be used to test the validity of the mass law for regeneration.

It requires several weeks for a leaf or stem to produce new shoots and roots of sufficient quantity to permit exact weighings. If the time allowed for regeneration is too short, so that the roots and shoots are too small, the error made in cutting off the roots

and shoots becomes considerable, since it is not possible to cut off these organs at their base with absolute accuracy. On the other hand, when the shoots reach a considerable size, they participate to an increasing degree in the assimilation. As long as their assimilating mass is small compared with the assimilating mass of the leaf on which they grow, the error committed in neglecting this influence is small; if necessary it can be corrected by deducting the mass of the shoots from that of the leaf in calculating the influence of the active mass of the leaf on the production of roots or shoots. The writer carried on his experiments for about three or four weeks; in this time the mass of roots and shoots produced under the conditions of temperature and light prevailing in the greenhouse was sufficiently large to make the error committed in cutting off the roots and shoots comparatively small. In order to rule out accidental variations, each experiment was made on a larger number of leaves or stems, rarely fewer than six in one experiment.

Since the amount of water in leaves, roots, and shoots is variable, it was impossible to base conclusions on measurements of the fresh weight of the mass of roots or shoots produced and the dry weight had to be used instead. The dry weight was determined after the organs had been kept in a drying oven for about 24 hours at a temperature of about 100°C. Tests showed that this sufficed to give a constant dry weight.

Having stated these preliminaries we may now begin to give the data which show that the production of roots and shoots in sister leaves is in proportion to the active mass (or the dry weight) of the sister leaves.

CHAPTER III

REGENERATION AND MASS RELATION IN ISOLATED LEAVES OF *BRYOPHYLLUM*

It will be shown, first, that equal masses of sister leaves of *Bryophyllum calycinum* produce, in equal time and under equal conditions of illumination, temperature, moisture, and chemical environment, equal quantities of shoots and roots.

Experiment 1.—Seven pairs of sister leaves of equal size were detached from stems and were suspended in an aquarium so that their apices dipped in water (Fig. 14). The experiment lasted from Mar. 20 to Apr. 11, 1923. From the notches near or in the water new roots and shoots arose. It was expected that each leaf of a pair would produce the same mass of roots and shoots from the notches, or that the 7 leaves, 1 each from a pair, would produce the same mass of roots and shoots as their 7 sister leaves, per gram dry weight of leaves. This was found to be approximately correct. The roots and shoots were removed from the leaves after 22 days, and leaves, roots, and shoots were dried for 24 hours in an electric oven at about 100°C. The two sets of sister leaves are designated as Set I and Set II in Table I.

TABLE I

	Dry weight of leaves, grams	Dry weight of shoots regenerated, grams	Dry weight of roots regenerated, grams	1 gm. dry weight of leaves produced	
				Shoots, milligrams	Roots, milligrams
Set I	1.528	0.405	0.153	265	100
Set II	1.665	0.464	0.166	278	100

Each of the two sets of 7 leaves produced therefore approximately equal masses of dry weight of shoots and roots per gram dry weight of leaves.

Experiment 2.—Nineteen pairs of sister leaves were used, one leaf of each pair (designated as 2 in Fig. 15) was left intact, while each sister leaf was cut into a small apical and a larger basal piece (1*a* and 1*b*). All dipped with the apical end into water (Fig. 15). The figure shows that the roots and shoots produced by sister

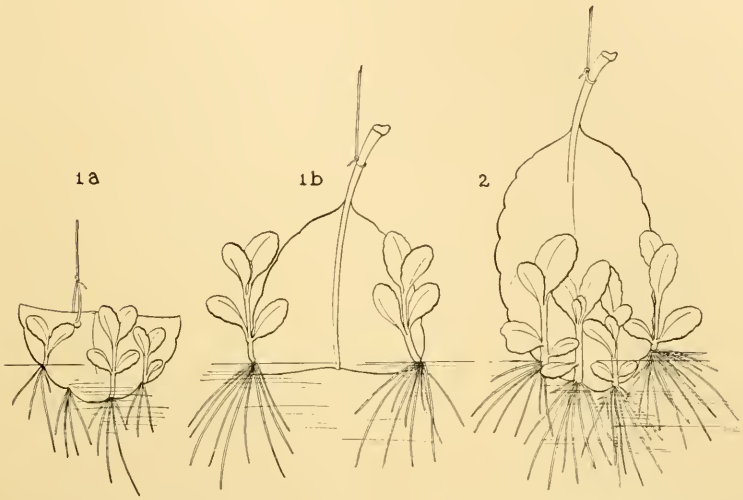

FIG. 15.—One leaf (2) intact, the sister leaf cut into 2 pieces, a small apical one (1*a*), and a larger basal one (1*b*). Shoot and root production are in proportion to the mass of the pieces. Mar. 26 to Apr. 17, 1923.

leaves varied approximately in proportion to the mass of the pieces of the leaves. This was confirmed by the measurements in Table II. The experiment lasted from Mar. 26 to Apr. 17, 1923. The 19 whole leaves are termed Set II, the 19 sister leaves each cut into two pieces, 1*a* and 1*b*, are termed Set I.

TABLE II

	Dry weight of leaves, grams	Dry weight of shoots regenerated, grams	Dry weight of roots regenerated, grams	1 gm. dry weight of leaves produced	
				Shoots, milligrams	Roots, milligrams
Set I *a*	1.751	0.409	0.095	234	54
Set I *b*	4.384	0.872	0.248	199	57
Set II	6.060	1.216	0.349	201	58

The experiment shows that the mass (in dry weight) of shoots and roots regenerated by isolated sister leaves of *Bryophyllum* varies under equal conditions approximately with the mass (in dry weight) of the leaves. On the assumption that the mass of material produced in sister leaves under the influence of light varies with the mass of the leaves, it is suggested that the quantity of regeneration is determined by the mass of material produced in the leaf under the influence of light.

That the shoot production in the small apical pieces (1*a*) is comparatively a little greater than in the large pieces is probably

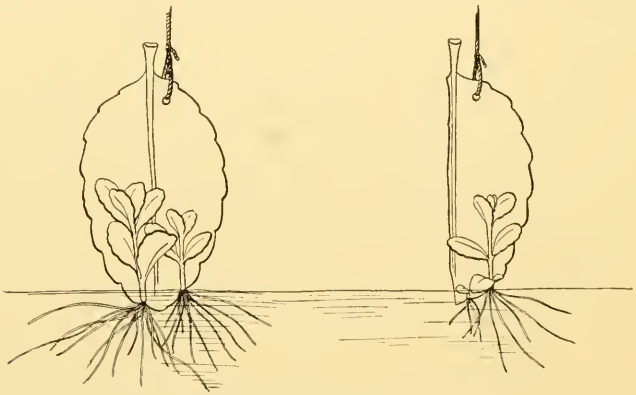

FIG. 16.—Root and shoot formation in whole leaf and in sister leaf (from which the left half is cut off) dipping with their apices in water. Diagrammatic.

due to the fact that the sap has not so far to travel to reach the notches where growth occurs in the small pieces (1*a*) as in the larger pieces of leaf (2 and 1*b*), and that comparatively more of the material available in a piece of leaf can go into the new roots and shoots when the piece is small than when it is large.

Experiment 3.—One set of 6 isolated leaves (Set II) remained intact while the mass of each leaf of the other set was reduced to approximately one-half by cutting away one side of each leaf (Set I, Fig. 16). The masses of the two sets of leaves were therefore no longer equal but had approximately the ratio of about 1:2. It was to be expected that the dry weights of the shoots and roots produced by the two sets of leaves should also be in the ratio of about 1:2, and this was the case (within the limits of the possible accuracy of such experiments) (Table III). One gram dry weight of leaves produced within the limits of the

accuracy of the experiments equal quantities of dry weight of roots and shoots. The tips of the two sets of leaves dipped into water, and roots and shoots developed only in the submerged notches (Fig. 16).

TABLE III.—DURATION OF EXPERIMENT, 30 DAYS

	Dry weight of			1 gm. dry weight of leaves produced	
	Leaves, grams	Shoots, grams	Roots, grams	Shoots, milligrams	Roots, milligrams
Set I. Six half leaves....	1.245	0.174	0.054	140	43
Set II. Six whole leaves..	2.300	0.283	0.092	123	40

Experiment 4.—Leaves were suspended sidewise, their lower edges dipping in water (Fig. 17). In both sets of leaves the upper edge was cut off; in one set the lower edge with the exception of one or two notches was cut off (Set I), while in the other set (Set II) all the notches of the lower edge were left intact. This was

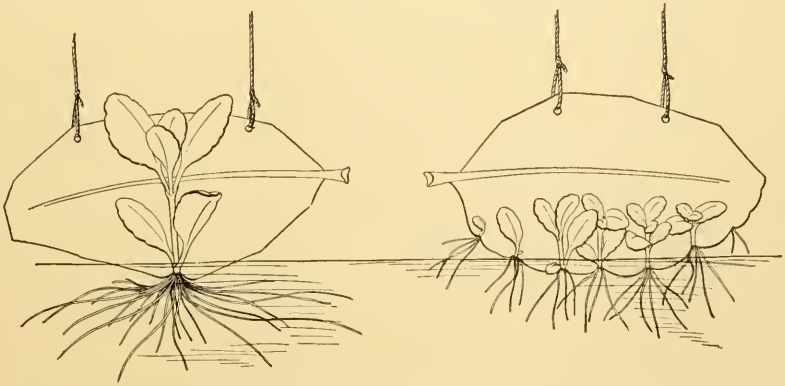

FIG. 17.—Sister leaves suspended sidewise. The upper edge is cut off in both leaves. In leaf to left, all notches except one removed; in leaf to right, all notches on the lower side preserved. Duration of experiment 33 days. After nature.

done to make the number of shoots produced by both sets unequal and to show that the general law enunciated remains true, even if the number of shoots produced in the two sets of leaves differs considerably. Thus, the leaves of Set I produced 6 and the leaves of Set II produced 16 shoots, yet the mass of the two sets of shoots was approximately the same, although it seems that when more shoots develop in one leaf the available material

furnished by the leaf can be utilized more fully than when all of the material goes into one shoot. This is indicated in the figures of Table IV.

TABLE IV.—DURATION OF EXPERIMENT, 33 DAYS

	Dry weight of			1 gm. dry weight of leaves produced	
	Leaves, grams	Shoots, grams	Roots, grams	Shoots, milligrams	Roots, milligrams
Set I. Five leaves with one or two notches....	1.810	0.248	0.106	137	59
Set II. Five sister leaves with more notches.....	1.778	0.270	0.121	152	68

On the basis of these and numerous similar experiments we can enunciate the following law:

Equal masses of sister leaves of *Bryophyllum calycinum* produce, in equal time, under equal conditions of temperature, moisture, illumination, and aeration, approximately equal masses of roots and shoots, regardless of the number of shoots or roots formed (except that a comparatively larger number of shoots may possibly permit a more complete utilization of the material furnished by the leaf than if only one shoot is formed). When the masses of two sister leaves are unequal, the masses of shoots and roots produced by them are, within the limits of the accuracy of the experiment, directly proportional to the masses of the leaves.

To complete the proof that we are dealing here with the action of the quantity of material produced by assimilation it remains to be shown that the results described occur only in the presence of light, while in the dark the production of roots or shoots in an isolated leaf of *Bryophyllum* is negligible. The slight regeneration in the dark occurs at the expense of material which was contained in the sap at the time the leaf was placed in the dark.

Twelve pairs of sister leaves were used, all of which were suspended so that their apices dipped into water as in Fig. 18. One leaf (a_1) of each pair was exposed to scattered daylight, while the 12 sister leaves (a) were kept in the dark, but all at the same temperature. The experiment lasted from Mar. 13 to Apr. 4. The total mass of shoots and roots produced in the dark was less than 14 per cent of that produced in the light during the same time (Fig. 18 and Table V).

We may, therefore, draw the conclusion that the quantity of regeneration of a detached leaf of *Bryophyllum* (measured

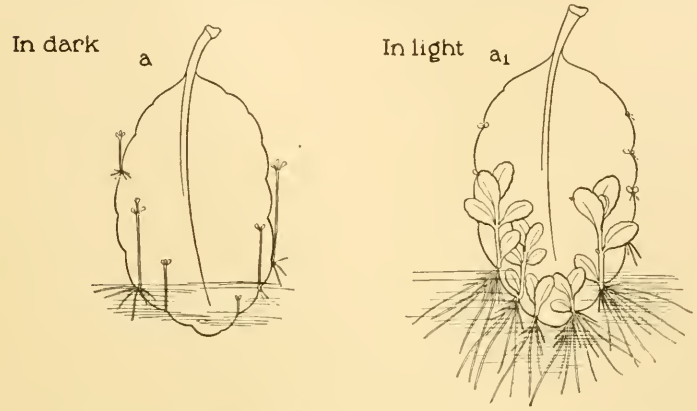

FIG. 18.—Influence of light on mass of shoot and root production from detached leaves, one leaf *a* kept in dark, sister leaf a_1 kept in light. The mass of shoot and root production in the dark is only a small fraction of that in light. Mar. 13 to Apr. 4, 1923.

in dry weight of regenerated organs) under the influence of light is determined chiefly by the mass of material produced by assimilation.

TABLE V

	Dry weight of leaves, grams	Dry weight of shoots regenerated, grams	Dry weight of roots regenerated, grams	1 gm. dry weight of leaves produced	
				Shoots, milligrams	Roots, milligrams
Set a_1 (in light).....	3.335	0.838	0.288	251	86
Set *a* (in dark).....	2.445	0.102	0.008	42	3

Not all the material formed under the influence of light in a leaf is utilized for the formation of roots or shoots; part of the material may be used for the growth of the leaf itself. This was shown in the following way:

Six pairs of sister leaves were cut off from plants. Each of the two sets of leaves had almost equal wet weight at the beginning. Six leaves were put into the drying oven at once to determine the dry weight at the start of the experiment. The 6 sister leaves were suspended in an aquarium, their apices dipping into water. At the apex, regeneration took place. Twenty-nine days later, after a considerable mass of roots and shoots had developed,

their dry weight as well as that of the leaves was determined. It was found that the dry weight of the leaves themselves (not including the dry weight of the shoots and roots formed) was considerably higher than was that of the sister leaves at the beginning of the experiment. The increase in the dry weight of the leaves was less, however, than the dry weight of shoots and roots produced by the leaves. Three sets of experiments were performed. In these three sets the leaves gained in dry weight 612 milligrams. The dry weight of shoots formed was 1.200 grams, and the dry weight of roots formed was 352 milligrams. Hence two and a half times as much material was utilized for the formation of new shoots and roots as for the growth of the leaf. Yet it is of importance that not all of the material available in the leaf for growth was used for regeneration.

TABLE VI.—DURATION OF THE THREE SETS OF EXPERIMENTS, JUNE 1 TO 30, 1920

I. *Six pairs of sister leaves.*	
a. Six leaves, fresh weight, 25.385 grams, dry weight, June 2	2.259 grams
b. Six sister leaves, fresh weight, 24.120 grams, dry weight, June 30	2.404 grams
Increase in dry weight of leaves in *b*	145 milligrams
Dry weight of shoots formed in *b* June 30	320 milligrams
Dry weight of roots formed in *b* June 30	102 milligrams
II. *Seven pairs of sister leaves.*	
a. Seven leaves, fresh weight, 28.490 grams, dry weight, June 2	2.674 grams
b. Seven sister leaves, fresh weight, 27.040 grams, dry weight, June 30	2.849 grams
Increase in dry weight of leaves in *b*	175 milligrams
Dry weight of shoots formed in *b* June 30	383 milligrams
Dry weight of roots formed in *b* June 30	92 milligrams
III. *Eight pairs of sister leaves.*	
a. Eight leaves, fresh weight, 28.825 grams, dry weight, June 2	2.552 grams
b. Eight sister leaves, fresh weight, 28.500 grams, dry weight, June 30	2.844 grams
Increase in dry weight of leaves in *b*	292 milligrams
Dry weight of shoots formed in *b* June 30	497 milligrams
Dry weight of roots formed in *b* June 30	158 milligrams

This explains in part why in the normal plant no regeneration of roots and shoots occurs in the leaf. In the normal plant all material is used either for growth of the leaf or for growth of the stem, apex, roots and stem itself, as will be seen later.

CHAPTER IV

THE INHIBITING ACTION OF THE RAPIDLY GROWING NOTCHES ON THE OTHER NOTCHES OF A LEAF

In Figs. 14 to 17 it is obvious that only those notches of a leaf which dip into the water give rise to new growth of shoots and roots, while in the other notches no growth is noticeable. This is not so at the beginning of the experiment; at first, growth of tiny roots and even of shoots may commence in many notches of the leaf, but soon the growth stops in all the notches except those dipping into water or which are close to the water. When

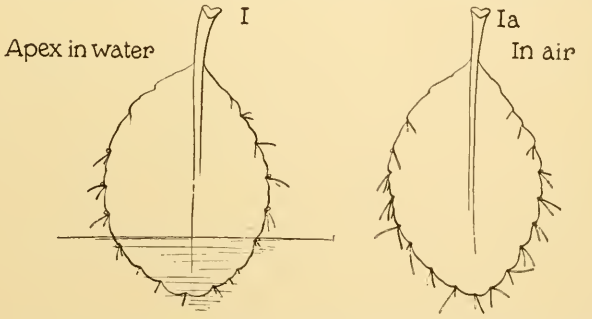

FIG. 19.—I and I*a*, sister leaves; I, dipping with the apex into water; I*a*, suspended entirely in moist air. Formation of roots and shoots commences in many notches in both leaves simultaneously. Drawn after 12 days.

the roots or shoots in these latter notches begin to grow, the tiny air roots already formed in the other notches shrink, dry out, and disappear; and the buds in the notches which upon further growth would have developed into shoots stop growing, so that after a few weeks no further growth is noticeable, except that in the notches which are in the water or near the edge of the water. This can be easily illustrated with large leaves or with the succulent leaves as they develop in the greenhouse during the winter. Figure 19 represents such an experiment. On Feb. 12, 1921, two sets of sister leaves were cut off and one of each pair was suspended entirely in air, while the other dipped with the apex into water. Figure 19 shows the appearance of the leaves on the

twelfth day. Both leaves produced tiny roots in almost all notches, both in air (I*a*, Fig. 19), and in water (I, Fig. 19). This, however, soon changed, as Fig. 20 shows, which was drawn of the same two leaves 6 days later (Mar. 1). Leaf I, the apex of which dipped into water, produced rapidly growing shoots and roots in water, and the roots formed in the beginning in the upper notches of this leaf had already dried out and soon disappeared; the shoots in the notches some distance from the water had also stopped growing (I, Fig. 20). In the other leaf, (I*a*, Fig. 20) which was entirely in air, all the roots still continued to grow and also a number of shoots, but much more slowly than

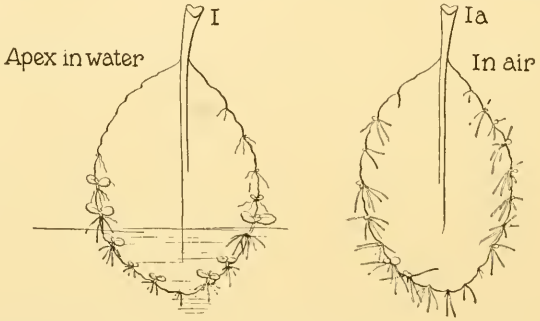

Fig. 20.—Same leaves as in Fig. 19, drawn 6 days later. In leaf I, the roots and shoots continue to grow only in the notches in or near the water while those farther away from the water dry out. In leaf I*a*, the roots and shoots in all the notches still continue to grow.

those at the apex of the other leaf. It is, therefore, obvious that the wilting of the air roots of leaf I, the apex of which dipped into water, must have been due to the fact that the more rapid growth of the shoots and roots in the notches under water attracted all or most of the sap of the whole leaf to these notches, thereby preventing the growth in the other notches and causing the air roots already formed in these notches to wilt. These results are quite general, the only variation being that when the leaf is small or less fleshy the roots and shoots may from the beginning be more exclusively confined to that part of the leaf which dips into water. When the leaf is entirely in air, roots and shoots grow out in almost all the notches, but the growth is always slower than in the notches dipping into the water.

It is therefore a general rule that the more rapidly growing notches of a leaf will attract all or almost all the sap from the

whole leaf to themselves, thereby stopping the growth in the rest of the notches of the leaf. Since this rule is of fundamental importance in regeneration, it was necessary to furnish a quantitative proof.

Thirteen pairs of sister leaves were used for the experiment, one leaf of each pair dipping with its apex into water while the

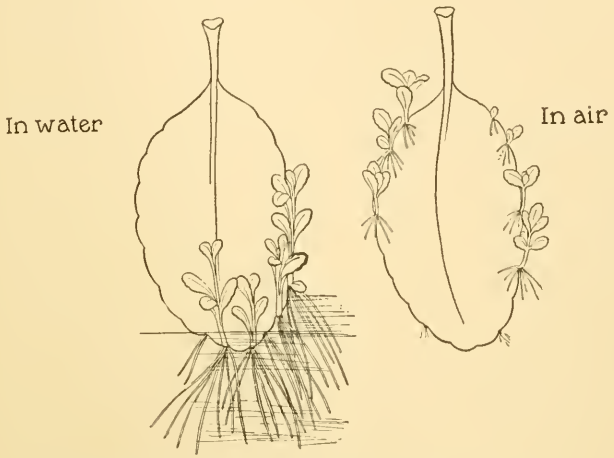

FIG. 21.—Difference in the place of regeneration when the leaf is suspended entirely in air and when its apex dips into water.

sister leaf was suspended entirely in air. Table VII shows that the mass of shoots and roots produced in the leaves dipping into water was considerably greater than the mass of shoots and roots produced simultaneously and under equal conditions by the sister leaves suspended in air. Figure 21 shows the difference in the appearance of the two leaves.

TABLE VII

	Dry weight of leaves, grams	Dry weight of shoots regenerated, grams	Dry weight of roots regenerated, grams	1 gm. dry weight of leaves produced	
				Shoots, milligrams	Roots, milligrams
Thirteen leaves dipping in water	1.943	0.524	0.123	270	63
Thirteen leaves suspended in air	1.909	0.322	0.051	169	27

It is obvious that the leaves suspended in air form a much smaller quantity of dry weight of shoots and roots per gram of dry weight of leaf during the same time and under the same conditions than the leaves dipping into water. Hence, if we accelerate the growth of some notches in the leaf, *e.g.*, by dipping them into water, we thereby inhibit the growth in the other notches.

When leaves are suspended entirely and permanently in air, practically all the notches commence to form shoots and roots

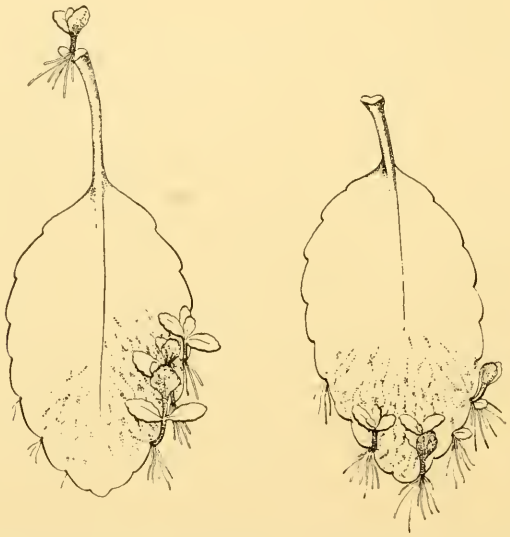

FIG. 22.—Leaves with purplish pigment. The pigment from the whole leaf migrates to the notches where new shoots are growing.

(see Fig. 20), but not all will continue to grow. Some notches will grow more rapidly than others and all the material will flow to the more rapidly growing notches. This explains why ultimately only a limited number of notches will continue to grow in air, usually in the more fleshy parts of the leaf (leaf to right in Fig. 21).

The fact that the sap available in a leaf flows to those notches where the growth is most rapid can be seen directly in leaves which form a purplish pigment (probably anthocyanin). This occurs only in leaves of *Bryophyllum* suspended in air, not in leaves which dip into water. Figure 22 shows two leaves of this kind suspended in moist air. The experiment lasted from Feb.

17 to Apr. 5. The anthocyanin is indicated by stippling. In both leaves the anthocyanin goes into the new shoots, collecting in that part of the leaf where the notches give rise to new growth.

These results then establish the principle that when the growth of shoots and roots is accelerated in certain notches of a leaf the sap from the whole leaf has a tendency to flow to these notches, as a consequence of which the growth in the other notches is inhibited. We shall see later that this principle not only holds for regeneration in the leaf but also in the stem.

CHAPTER V

THE INFLUENCE OF GRAVITY ON THE FORMATION OF ROOTS AND SHOOTS IN A DETACHED LEAF OF *BRYOPHYLLUM*

The rule discussed in the preceding paragraph that all the sap of a detached leaf flows to those notches where the growth is more rapid explains in a simple way one of the mysterious phenomena in the regeneration and growth of plant organs; namely, the influence of gravity on regeneration.

This influence can be demonstrated and studied in leaves of *Bryophyllum* which are suspended entirely in air in a vertical

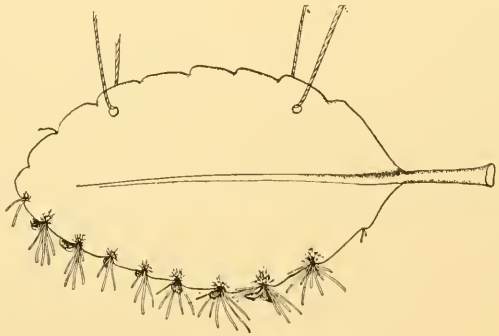

FIG. 23.—Influence of gravity on formation of roots and shoots in leaves suspended sidewise and in a vertical plane in moist air. Roots and shoots are formed only on the lower edge of the leaf.

plane and sidewise (Fig. 23). In this case shoots and roots develop chiefly or exclusively on the lower edge of the leaf, not on the upper edge. This is a striking and universal result. (The figure was drawn on the eighteenth day.) It can be shown that roots and shoots can develop also on the upper side of such a leaf; all that is necessary for this purpose is to cut off the lower edge of leaves suspended sidewise in moist air. In this case, a vigorous development of shoots takes place in the upper notches of the leaf. The two leaves in Fig. 24 are sister leaves, both suspended vertically and sidewise in moist air. The leaf on the

left was intact while the lower edge of the leaf on the right was cut off. The intact leaf on the left formed roots and shoots almost exclusively on the lower side, the sister leaf only on the upper side. The drawing was made on the thirty-third day of the experiment.

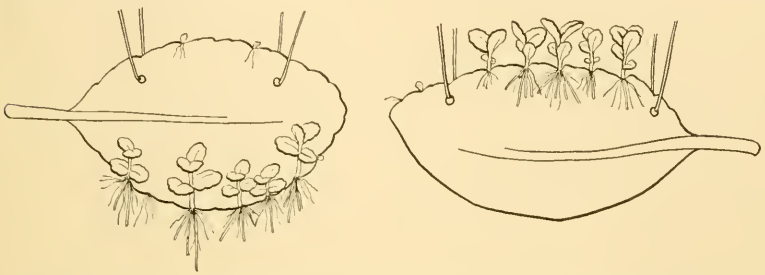

FIG. 24.—Sister leaves suspended sidewise in moist air. Left leaf intact, while the lower edge is cut off in leaf to right. In left leaf shoots and roots develop on lower edge, and the rapid development of roots and shoots on the lower side retards or inhibits the development of roots and shoots on the upper side of the leaf. In leaf to the right, where the lower edge is cut off, the development of roots and shoots on the upper edge is neither restricted nor retarded.

The explanation is as follows: The effect of gravity consisted in a greater collection of sap on the lower edge of the fleshy parts of the leaves. As a consequence the growth on the lower edge of leaves suspended sidewise is accelerated and this now leads sec-

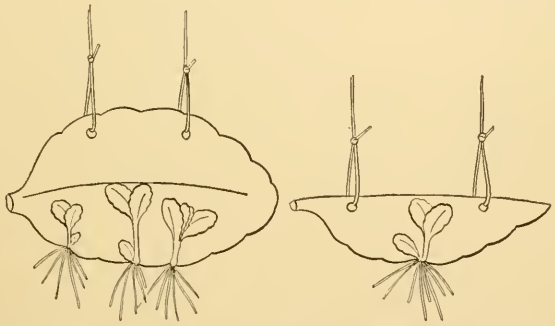

FIG. 25.—Quantitative difference in regeneration when a whole leaf and a half leaf are suspended sidewise in air.

ondarily to a flow of the sap of the whole leaf to the lower edge. This suppresses the growth in the upper notches. If the lower edge of a leaf suspended sidewise in moist air is cut off a collection of sap will also occur in the lower edge, but since no growth can occur there it will *not* result in the flow of the sap of the whole

leaf to the lower edge of the leaf. Hence nothing prevents the shoots in the upper edge from growing out. As soon as this happens all the sap from the leaf will flow to the notches of the upper edge. The correctness of the explanation of the results in Fig. 24 can be proved quantitatively.

A number of pairs of sister leaves were suspended vertically and sidewise in air, one leaf of each pair remaining intact, while the upper half of the sister leaf was cut away (as shown in Fig. 25). The whole leaves formed practically twice as great a mass of shoots and roots on the lower edge as the half leaves and this difference is noticeable in Fig. 25. Thirteen whole leaves and their thirteen half sister leaves were chosen for comparison in Table VIII. The experiment lasted from May 8 to 29.

TABLE VIII

	Dry weight of leaves, grams	Dry weight of shoots, grams	1 gm. dry weight of leaves produced dry weight of shoots, milligrams
Thirteen whole leaves	2.174	0.410	188
Thirteen half sister leaves	1.118	0.235	209

The result proves that the excess regeneration in the *whole* leaves was produced by material furnished by both the lower and the upper half of the leaves, regeneration in the upper half being as a consequence impossible, since almost all the material available for regeneration in the upper half was consumed for regeneration in the lower half.

In the case just discussed, the regeneration in the lower half of the leaf is accelerated, since owing to the action of gravity the liquid in the leaf collects in the lower half. This happens when the leaf is suspended sidewise in the air but only rarely when it is suspended in the air with the apex down. The reason for this difference is probably that the apex of the leaf is very thin in comparison with the fleshy middle part of the leaf so that under the influence of gravity liquid cannot collect as abundantly in the apex of the leaf as in the lateral parts.

This leads to a new conception of the nature of the influence of gravity on the formation of organs in plants. All that gravity does and need do is to cause a collection of the sap of a plant

organ in the lowest parts of the organ. The water of the sap has the effect of accelerating the growth in the lower part where the sap collects and this causes secondarily a flow of sap of the whole organ to the lower edge. The inhibition of growth in the upper edge of the organ, therefore, is not a direct effect of gravity but only a secondary consequence. This can be proved by suspending detached leaves sidewise under water but near the surface. In this case a seepage of tissue fluid to the lower edge of the leaf will also occur, but since the upper notches are in water, the rate of chemical reaction in the notches on the upper side of the leaf

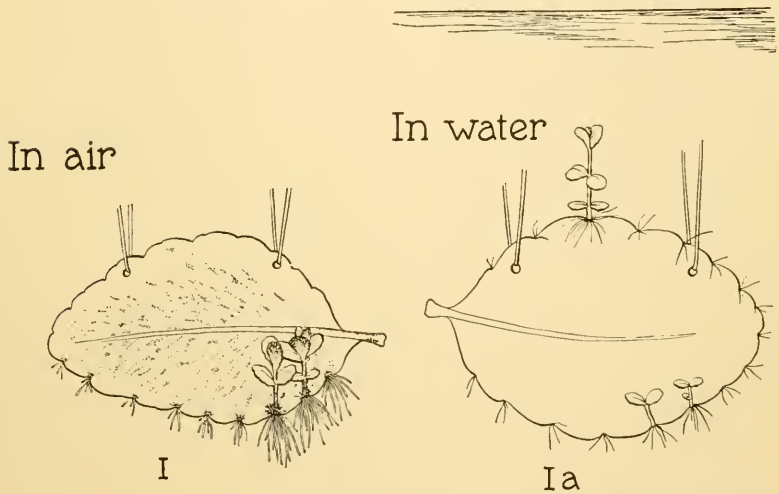

FIG. 26.—In leaf I, suspended in air, roots and shoots are formed only in the notches of the lower side of the leaf, while in leaf I*a*, suspended in water, this directive influence of gravity does not exist, inasmuch as roots and shoots are formed on both the upper and the lower sides of each leaf. Only the leaf suspended in air shows the red pigment indicated by stippling. Oct. 26 to Dec. 15.

will not be slower than in the notches of the lower side of the leaf. Figure 26 shows such an experiment. Leaf I was suspended sidewise in air and roots and shoots formed in the lower notches exclusively. The sister leaf I*a* was suspended sidewise under water near the upper surface of the water. In this case the influence of gravity disappeared completely since the formation of roots and shoots in the upper notches was as plentiful as in the lower notches.

It seems as if it were necessary to distinguish in the plant between the liquid enclosed in vessels (resembling the blood and

lymph of animals) and the liquid contained in the spaces between tissues and cells, the tissue sap. This latter sap can be caused to collect in the lowest parts of the leaf under the influence of gravity, while the sap of plants contained in vessels probably cannot be caused by gravity to gather in the lowest parts. This will become clear by experiments described in later chapters.

It must strike every biologist as strange that gravity plays such a great role in organ formation in plants, while it is rare to find instances where the organ formation of animals is determined by gravity. The reason may be that in plants the tissue sap outside the vessels can move more freely than in animals, where the influence of gravity on the tissue sap can only be demonstrated in the case of edema.

CHAPTER VI

WHY DOES SEPARATION OF A LEAF FROM THE PLANT INDUCE THE GROWTH OF ROOTS AND SHOOTS IN THE LEAF?

We now come to the main problem of regeneration; namely, why mutilation leads to growth which would not have occurred without mutilation. In the case of the leaf of *Bryophyllum*, the question arises: Why does the leaf form new shoots and roots in its notches only when it is detached from the plant, but does not do so when connected with the plant? The quantitative method permits us to give a definite answer: that as long as the leaf is connected with the stem of a normal plant, the material formed under the influence of light in the leaf goes into the stem and causes growth of the stem as well as growth of shoots at the apex and of roots at the base of the stem.

It is not necessary that the leaf should be connected with a whole plant in order to inhibit the formation of shoots in the leaf. The inhibition of regeneration in a leaf is already noticeable when the leaf is attached to a small piece of stem.

Figure 27 represents a pair of sister leaves, one detached, the other in connection with a small piece of stem. Each leaf dips with its apex into water. The leaf without stem has formed four large shoots and roots, while the sister leaf, which is attached to a piece of stem, has just commenced to produce a tiny shoot.

The same inhibition can be demonstrated still more strikingly when the two leaves are suspended entirely in moist air (Fig. 28) (instead of dipping into water). The one leaf without stem had formed three shoots in notches, while no regeneration occurred in the sister leaf which remained in connection with a piece of stem. The small piece of stem suppressed all shoot and root formation in the leaf; but a shoot was formed on the stem in the axil of the leaf. The stem inhibited the shoot formation in the leaf because the material for regeneration went into the stem. These two leaves had the red pigment (anthocyanin?) which substance makes it possible to follow the path of migration of material.

In the leaf without stem the red pigment collected in the lower part where three shoots were formed, while in the leaf attached to a stem it collected near the petiole of the leaf.

In order to prove quantitatively that the inhibitory action of a piece of stem on root and shoot formation in a leaf is due to the

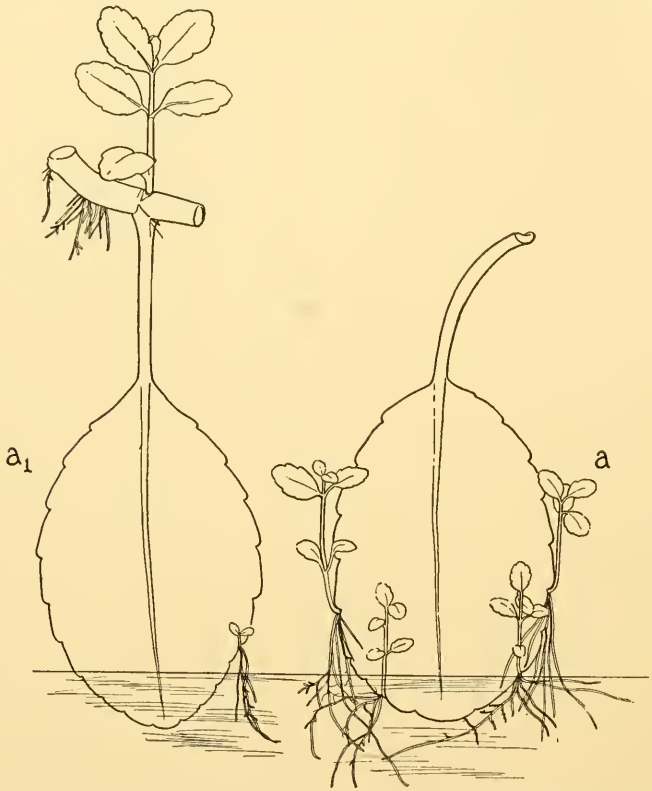

FIG. 27.—Sister leaves dipping with their apices in water. Leaf *a*, without stem, has formed 4 shoots in 4 different notches. Leaf a_1, attached to a piece of stem, has just commenced to form 1 tiny shoot in 1 notch. The material which caused shoot production in leaf *a* was utilized by the stem in leaf a_1 for the production of a large shoot on the stem, for callus formation at the basal end of the stem, and for a slight geotropic curvature. The drawing was made 7 weeks after the beginning of the experiment.

flow of material from the leaf into the stem the following method was used:

Small pieces of a stem of *Bryophyllum*, containing 1 node with 2 leaves each, were cut out from a plant. Each piece of stem was split longitudinally through the middle (Fig. 29), to make the

two pieces of half stem (b and b_1) as equal in mass as possible. In order to minimize the error in cutting a larger number of pieces of stem were used for one experiment. One half stem b (Fig. 29) was removed at once from one of each pair of sister

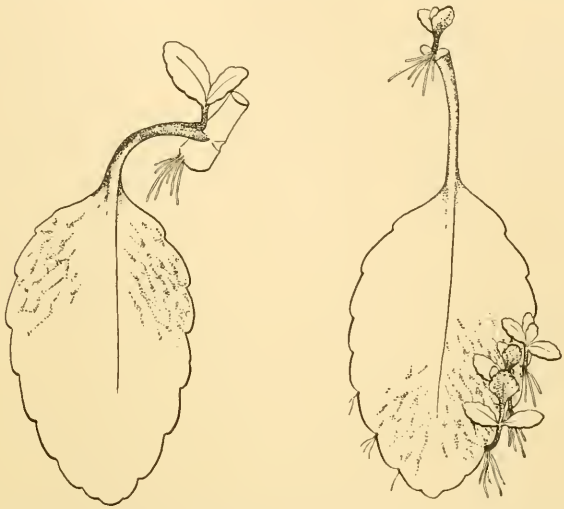

FIG. 28.—Two sister leaves suspended entirely in air. The stippling indicates the reddish pigment. In the leaf to the left, which is connected with a piece of stem, the pigment flows into the axillary shoot of the stem and into the petiole, and is collected in the upper part of the leaf nearest the petiole. In the sister leaf, without stem, it collects near the place where the new shoots are formed and in the new shoots. Duration of experiment, Feb. 17 to Apr. 5.

leaves, and the dry weight of these half stems (b) was determined immediately, the other half stem, b_1, remained in connection with the leaf, a_1. The two sets of sister leaves (a_1 with a half stem

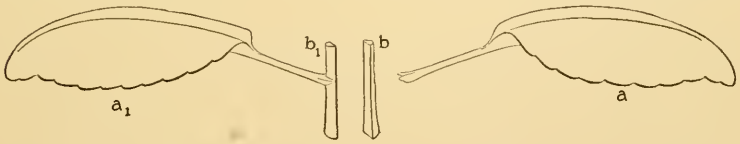

FIG. 29.—Method of proving that the dry weight of a piece of stem kept in connection with a leaf increases at the expense of the shoot and root production of the leaf which is correspondingly diminished (see text).

attached, the other a without a half stem) were suspended for several weeks in moist air, their apices dipping into water (Fig. 30). The leaves a_1, with a half stem (b_1) attached, formed a smaller mass of shoots and roots than the leaves a, without such

a piece of stem. At the end of the experiment, the dry weights of the leaves, shoots, roots, a and a_1, and of the shoots and roots produced by them, and of the half stems b_1, which had been left in connection with the leaves, were determined. It was found that the dry weight of the half stems b_1 (Fig. 30), left in connection with the leaves a_1, had increased and that this increase in weight was sufficient to account for the excess in the dry weight of roots and shoots formed in the leaves a, without pieces of stem. In other words, the inhibitory action of the stem on the regeneration in the leaf was due to the fact that the leaf sent part of its

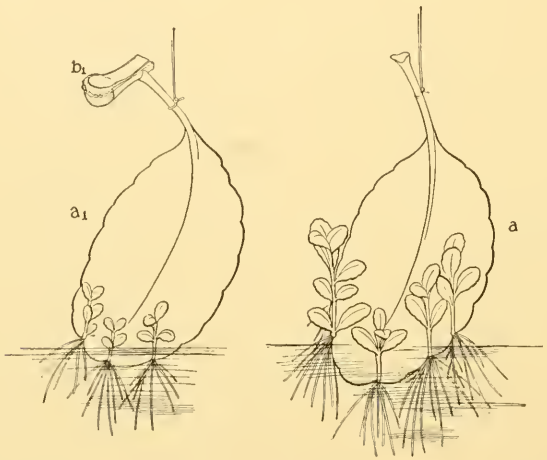

FIG. 30.—Leaf a_1, with a small piece of stem attached, produces a smaller mass of shoots and roots than the sister leaf a without stem. The piece of stem increases in mass, especially through callus formation, and this accounts for the inhibiting action of the stem on shoot and root formation in leaf. Axillary bud of stem removed. Apr. 6 to 26.

material into the stem, which otherwise would have been available for regeneration in the leaf.

In such experiments the axillary bud of the half stem left in connection with the leaf often grows out and it is natural to infer that this growth withdraws material from the stem. It can be shown, however, that the stem inhibits the regenerative growth in the leaf also if this axillary bud is removed at the beginning of the experiment; and that, in this case also, the inhibitory influence of the piece of half stem on regeneration is due to the fact that the leaf sends part of the material produced by assimilation into the stem. In the stem it is used chiefly for

callus formation at the basal end and for increase in thickness as well as for longitudinal growth of the piece of stem (Fig. 30, b_1). The data in Table IX show the inhibiting effect of the piece of half stem attached to the leaf on the quantity of regeneration in the leaf.

TABLE IX

	Dry weight of leaves, grams	Dry weight of shoots regenerated by leaves, grams	Dry weight of roots regenerated by leaves, grams
Set I (with stem attached to leaf)...	2.991	0.427	0.132
Set II (without stem).....	3.116	0.939	0.273

Nineteen pairs of sister leaves were used in the experiment. The pieces of half stem attached to one of each pair of sister leaves were about 25 millimeters long.

The total dry weight of roots and shoots regenerated by the leaves with a piece of stem was 559 milligrams while that of the roots and shoots regenerated by the leaves without stem was 1,212 milligrams.

Hence the presence of the small piece of stem in Set I (Fig. 30) diminished the quantity of regeneration in the 19 leaves of this set by 653 milligrams. (The correction for the slight difference in the mass of the two sets of leaves reduced this value to about 630 milligrams.)

The dry weight of the 19 half stems determined at the beginning of the experiment was 0.747 gram. The dry weight of the 19 half stems (which were left in connection with the leaves) determined at the end of the experiment was 1.213 grams. Hence the stems connected with the leaves gained 466 milligrams which is slightly less than the excess of mass of shoots and roots regenerated in the leaf without stem over that with stem, namely 630 milligrams. This difference is, however, accounted for by the fact that the mass of the pieces of half stem connected with the leaves was diminished at the beginning of the experiment by cutting out the bud on the stem in the axil of the leaf. To make sure that the bud was removed, part of the surrounding tissue had also to be cut out. It is also possible that in addition the mutilation of the stem near the connection with

the petiole of the leaf may have created a slight obstacle for the sap flow into the stem. In any case, we shall see that if the half stem is not mutilated in this way its gain in dry weight exceeds slightly the difference in the dry weight of shoots and roots produced in the detached leaf and its sister leaf attached to a stem. Since the gain in the mass of the stem must have occurred in this experiment through material furnished by the leaf, the inhibitory influence of the stem on the regeneration in the leaf is within the limits of accuracy of the experiments accounted for by the flow of material from the leaf into the stem.

On account of the fundamental character of these experiments the results of 5 similar sets of experiments are given in Tables X and XI. The 5 sets of experiments were made each with 12 pairs of sister leaves, and in these experiments the axillary bud was not cut out. In Table X is given the dry weight of the half stem at the beginning and the end of the experiment. The last vertical column gives the increase of the dry weight of the half stem connected with the leaf.

TABLE X.—DRY WEIGHT OF HALF STEMS AT BEGINNING AND END OF EXPERIMENT

Experiment No.	Duration of experiment, days	Dry weight of half stem		Increase in dry weight of half stem during experiment, grams
		At beginning, grams	At end, grams	
1	30	0.474	1.054	0.580
2	33	0.426	0.753	0.327
3	33	0.415	1.034	0.619
4	30	0.563	1.029	0.466
5	30	0.422	0.823	0.401
				Total....2.393

This increase in the dry weight of material of the half stems connected with the leaf (which included shoots formed in the pieces of stem) should be equal to or larger than the mass of shoots formed in the detached leaves (Table XI) if the inhibitory action of the stem on root and shoot formation is due to the fact that the material available for root and shoot formation in the detached leaves flowed into the stem. The dry weight of roots

and shoots formed in each set of leaves was measured in each set of experiments.

TABLE XI.—DRY WEIGHT OF ROOTS AND SHOOTS FORMED IN DETACHED LEAVES AND IN LEAVES CONNECTED WITH HALF STEMS IN THE SAME EXPERIMENT AS IN TABLE X

Experiment No.	Duration of experiment, days	1. Detached leaves		Total, grams	2. Leaves with half stems		Total, grams	Difference in dry weight of 1 and 2, grams
		Dry weight			Dry weight			
		Roots, grams	Shoots, grams		Roots, grams	Shoots, grams		
1	30	0.169	0.412	0.581	0.039	0.067	0.106	0.475
2	33	0.133	0.408	0.541	0.062	0.158	0.220	0.321
3	33	0.127	0.509	0.636	0.045	0.114	0.159	0.477
4	30	0.127	0.438	0.565	0.053	0.143	0.196	0.369
5	30	0.101	0.416	0.517	0.043	0.162	0.205	0.312
								Total...1.954

The dry weight of the material which was given off by the leaves to the pieces of stem attached to them was 2.393 grams, while 1.954 grams was the excess of the dry weight of roots and shoots formed in the leaves entirely detached from stems over the dry weight of roots and shoots formed in the leaves with stems attached. This proves that the inhibitory effect of the stem on root and shoot production in the leaf is due to the fact that the material available for regeneration in the leaf flows into the stem. The amount of material given by the leaves to the stem is roughly 20 per cent greater than the amount which is utilized in the excess production of roots and shoots in the detached sister leaves. This excess is accounted for by the fact, discussed in the preceding chapter, that not all the new material produced by assimilation in the detached leaf goes into the formation of roots and shoots in the leaf, but that about 33 per cent of this material is used for the growth of the leaves themselves. When a piece of stem is attached to a leaf, part of this reserve material may also go into the stem.

When the axillary bud of the half piece of stem grows out, which does not usually happen, the flow of material from the leaf into the stem is much more considerable; and, accordingly, as is shown in the following experiment the inhibitory influence of the stem on regeneration in the leaf is still more considerable.

Large numbers of pieces of stem, about 25 millimeters long, each with 1 node and possessing 2 leaves, were cut out and the pieces of stem were split longitudinally as nearly in the middle of the stem as possible (Fig. 31), so that each sister leaf was connected with half a piece of stem. In one set of leaves the axillary bud of the stem was removed (II, Fig. 31), while in the set of sister leaves the axillary bud was left intact (I, Fig. 31). The leaves of both sets were suspended in an aquarium, dipping with their apices into water. The axillary buds grew out in 11 of the half stems with leaves attached, and these 11 specimens and their sister leaves connected with half stems the axillary buds of which were

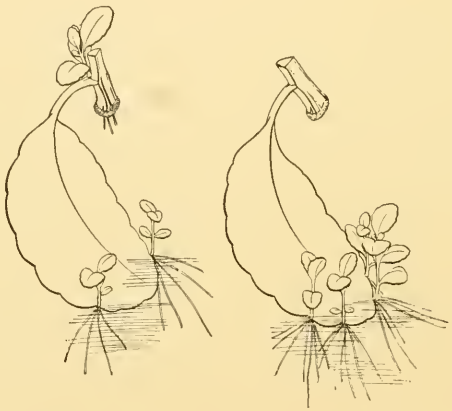

FIG. 31.—The inhibitory action of a piece of stem on root and shoot formation in leaf is greater when the axillary shoot can grow out as in I than in sister leaf II where the axillary bud of the stem was removed. Mar. 13 to Apr. 9.

removed, were selected for a quantitative measurement of the influence of the growth of the axillary bud on the regeneration in the leaf (Fig. 31). It was found that the leaves connected with the pieces of half stem, the axillary bud of which was cut out, produced a greater mass of shoots and roots than the sister leaves connected with the other piece of stem, the axillary bud of which was allowed to grow. This latter bud attracted and consumed a good deal of the material which in the sister leaves was available for regeneration in the leaves themselves. The experiment lasted from Mar. 13 to Apr. 9, 1923. Table XII gives the quantitative results.

TABLE XII

	Dry weight of leaves, grams	Dry weight of shoots regenerated by leaves, grams	Dry weight of roots regenerated by leaves, grams
Set I (with axillary shoot).....	1.745	0.056	0.027
Set II (without axillary bud).....	1.754	0.267	0.068

The dry weight of the two sets of leaves was about the same, but that of the roots and shoots produced in the leaves themselves was considerably smaller when the axillary bud of the stem was allowed to grow than when this was not the case. The

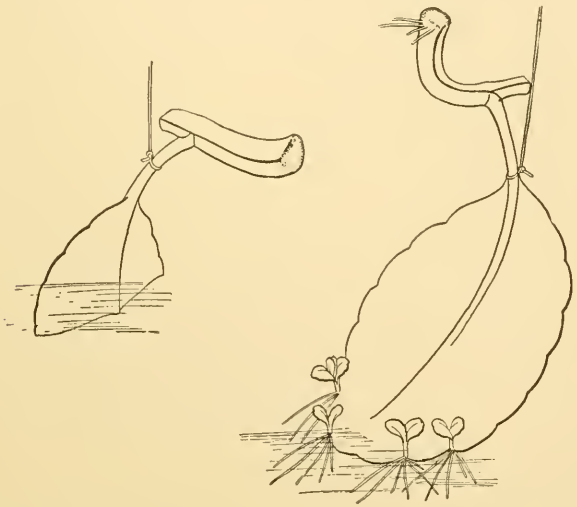

FIG. 32.—Sister leaves, leaf to the left reduced in size. In the small piece of leaf the stem inhibits the shoot and root formation completely, but in the larger leaf the same mass of stem fails to inhibit regeneration completely. Apr. 13 to 26, 1923.

leaves of Set I (Fig. 31), the stems of which formed axillary shoots, produced in all only 83 milligrams dry weight of roots and shoots, while the leaves of Set II (Fig. 31), the stems of which formed no axillary shoots, produced in all 335 milligrams of roots and shoots; *i.e.*, four times as much. This difference is accounted for by the weight of the 11 axillary shoots formed in the stems of Set I, which was 0.454 gram.

The inhibiting effect of a given piece of stem on regeneration in the leaf must also depend on the relative size of the leaf.

The material produced in a pair of sister leaves depends on their relative size. On the other hand, a piece of stem of a given size can in a given time consume only a limited quantity of material. Hence when a small piece of stem with 2 sister leaves is split longitudinally and the size of 1 leaf is reduced, the inhibitory effect of the piece of stem on regeneration in the leaf will be more complete in the reduced than in the whole leaf. Figure 32 shows that this is the case. On the other hand, when the piece of stem is very small, its inhibiting power on regeneration in the leaf approaches zero. This is illustrated in Fig. 33, where one

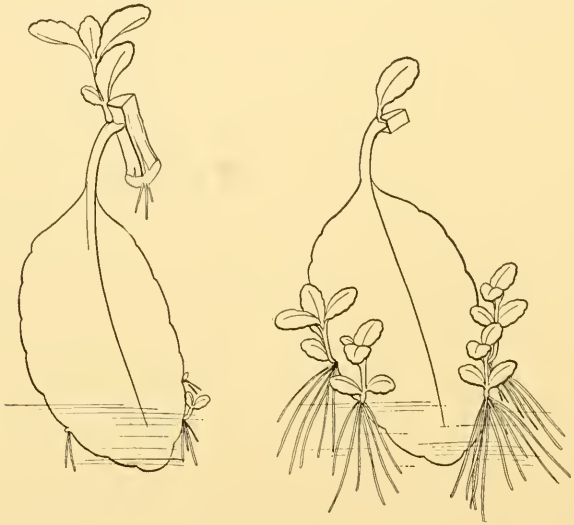

Fig. 33.—Sister leaves. The larger piece of stem inhibits the formation of shoots and roots in the leaf more completely than the smaller piece of stem.

leaf is connected with a piece of stem about 25 millimeters long, while the sister leaf had only a piece of stem about 4 millimeters long. The inhibitory effect of this small piece of stem was very slight compared with the inhibitory effect of the larger piece. When the piece of stem is sufficiently large the inhibitory effect on regeneration in the leaf may become complete.

In most of the experiments referred to in this chapter the apex of the leaf dipped into water. This is, however, not a necessary prerequisite; what has been said above about the inhibitory effect of a piece of stem on the shoot and root formation in a leaf remains true also when the leaves are suspended entirely in moist air, as shown in Fig. 28.

We have seen that the amount of shoot and root formation of a leaf in the dark is only a small fraction of the quantity of regeneration in the light. It was next of interest to find out whether there occurs also in the dark a diminution of the shoot formation in the leaf when a piece of stem is attached to it, and whether such an inhibition is accompanied in the dark also by a corresponding increase in the dry weight of a stem. It was found that the stems actually diminish or repress the already small amount of shoot formation of a leaf in the dark and that the dry weight

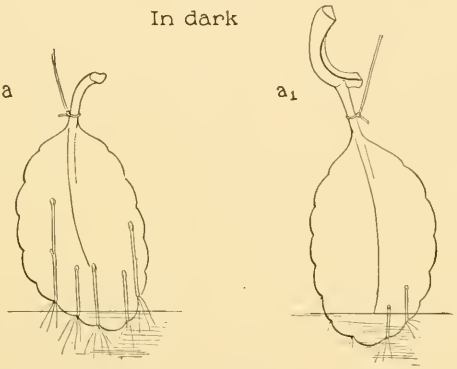

FIG. 34.—The inhibitory effect of the stem occurs also in the dark, leaf *a* producing a greater mass of shoots and roots than its sister leaf a_1, which has a piece of stem attached. Apr. 18 to May 9.

of the stems increases to about the same amount in weight as the shoot formation in the leaf is diminished. Figure 34 indicates the difference in the appearance of the leaves with and without stems attached when kept in the dark. The experiment lasted 20 days. The leaf *a*, without stem, produced more shoots than its sister leaf a_1 with a half piece of stem attached. The stems gained correspondingly in weight. The exact figures of the dry weight measurements of the experiment are given in Table XIII.

TABLE XIII

	Dry weight of leaves, grams	Dry weight of shoots regenerated by leaves, grams	Dry weight of roots regenerated by leaves, grams
Set *a*. Nine leaves without stem...	1.035	0.070	0.007
Set a_1. Nine sister leaves with pieces of half stems attached.....	1.027	0.006	0.004

The 9 leaves (a) without stems formed in all 77 milligrams of shoots and roots, while the sister leaves (a_1) with a piece of half stem attached, formed in all 10 milligrams of shoots and roots, a difference of 67 milligrams. The half stems had at the beginning a dry weight of 0.454 gram, while the half stems of a_1 had at the end of the experiment a dry weight of 0.505 gram, an increase of 51 milligrams. Hence the half stems in Set a_1 gained in the dark about enough in dry weight to account for the inhibitory effect of the stem of the leaf a_1 on regeneration in the leaf. In this experiment the stems formed no axillary shoot. In a repetition of the same experiment in the dark, in which 17 pairs of leaves with half stems were used, 7 of these leaves in which the stems remained attached formed axillary buds. As a consequence more material flowed from the leaves into the stems and the inhibition of shoot and root formation in the leaves with half stems attached became even more complete. Table XIV gives the results. The experiment lasted 22 days.

TABLE XIV.—DARK EXPERIMENT

	Dry weight of leaves, grams	Dry weight of shoots regenerated by leaves, grams	Dry weight of roots regenerated by leaves, grams
Set *a*. Seventeen leaves without stems	1.740	0.161	0.019
Set *b*. Seventeen sister leaves with pieces of half stems attached	1.732	0.005	0

The leaves without half stems produced in all 175 milligrams more dry weight of shoots and roots than the leaves with half stems attached, in which the inhibition of root and shoot formation was almost complete. The dry weight of 17 half stems at the beginning was 0.571 gram, at the end 0.879 gram. This latter figure included the weight of 7 axillary shoots. Hence the dry weight of the stems increased by 308 milligrams, more than enough to account for the inhibitory action of the stems on shoot and root production in the leaves with half stems attached.

In these experiments the material sent by the leaf into the stem in the absence of light had been formed previously by the light. The regeneration of plants in the dark resembles the regeneration

in starving animals, where the regeneration depends also on the hydrolysis of stored material.

We now understand why the leaf of *Bryophyllum calycinum*, when it is detached from the plant, forms shoots and roots in its notches while this regeneration is inhibited when the leaf forms part of a normal plant. The leaf connected with a normal plant can be dipped into water without forming roots or shoots in its notches. All the material which might be available for shoot and root formation in the leaf is sent into the stem. During a recent visit in Bermuda, I have had a chance to examine thousands of plants of *Bryophyllum calycinum* without finding a single case where a leaf connected with a plant had formed roots or shoots. The same has been true in my greenhouse, and only recently have I had an opportunity to observe about six plants, the older leaves of which formed some tiny shoots. The plants in which this occurred were old and in two boxes containing no other plants; so that the suspicion is justified that their roots had suffered some common injury or disease. When a stem contains many leaves, and when the growth of the stem is stopped or when the sap flow has suffered, it is possible that shoots and roots may originate on leaves still connected with the stem. All that is needed for such growth is that the flow of material from the leaf into the stem should be partially or completely prevented.

The fact that in such cases regeneration can occur in leaves connected with a stem, and hence without injury, eliminates the idea that "wound hormones" or "wound stimuli" are the cause of shoot and root formation in the notches of a detached leaf of *Bryophyllum*, as has already been stated in an earlier chapter.

This then solves the first part of the problem of regeneration; namely, the correlation of the new growth with the mutilation, and the solution is this, that, as a consequence of the mutilation the sap (that is the water and the solutes it contains) collects in places where it could not have collected without the mutilation. Only the quantitative method of experimentation made it possible to prove this correlation.

CHAPTER VII

THE VALIDITY OF THE MASS RELATION FOR REGENERATION IN A DEFOLIATED PIECE OF STEM OF *BRYOPHYLLUM CALYCINUM*

1. Experiments on Short and Long Pieces of Stem.—In experiments on the stem we encounter the second problem of regeneration; namely, its polar character. Regeneration has not infrequently a polar character; that is, different organs are formed at the opposite poles of a fragment from a mutilated organism (Figs. 5, 7, 9 and 11). While the polar character of regeneration is not uncommon, it is not universal. Thus in the case of regeneration in the leaf of *Bryophyllum* we do not notice that shoots arise from the notches at one end of the leaf while roots arise from the notches at the opposite end, but we notice that both roots and shoots arise always from the same notch, the formation of roots preceding that of shoots. It is quite possible that one side of the embryonic tissue of the notch (possibly the dorsal) gives rise to the root and the other (possibly the ventral) to the shoot, and if this is true it is also a case of polar regeneration. The polar character of regeneration in a defoliated piece of stem of *Bryophyllum* is, however, different since shoots and roots originate at a great distance from each other, shoots growing from the most apical node of the stem and roots from the base (Fig. 35). The problem is, What causes this specifically polar character of regeneration in the stem? Bonnet and Sachs[1] tried to explain this polarity on the assumption of a chemical difference between the ascending and descending sap; the latter being supposed to contain specific root-forming substances and the former specific shoot-forming substances. These specific organ-forming substances are as yet purely hypothetical though they may actually exist. If they exist they must be present everywhere in the sap of the leaf since every notch of an isolated leaf can give rise to both roots and shoots. We shall not deal with the problem of polarity

[1] Sachs, J.: *Stoff und Form der Pflanzenorgane, Arbeiten d. bot. Inst. in Würzburg*, vol. ii, pp. 452, 689, Leipzig, 1882.

in this and the next chapter but intend to show first that the same laws or rules which determine regeneration in the leaf suffice, to explain also regeneration in the stem. We will first prove that the quantity of regeneration of a piece of stem exposed to light is in direct proportion with the mass of the stem.

Each node of the stem of *Bryophyllum* has 2 dormant buds capable of growing into shoots. When a piece of defoliated stem

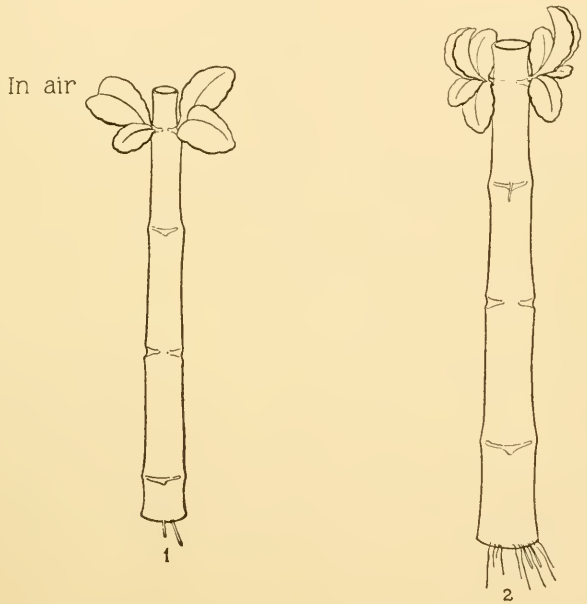

FIG. 35.—Two pieces of stem from the same plant, (1) apical, (2) basal. Suspended in moist air, shoots formed only in the apical node, roots at the base. The mass of shoots and roots is larger in the basal piece (2) which has the larger mass. Oct. 4 to Nov. 7.

is cut from a plant and suspended in moist air only the 2 buds of the most apical node will grow into permanent shoots, while the buds in all the nodes below will remain dormant (Fig. 35). Permanent roots will grow only at the base of each piece, though transitorily air roots may begin to form in any node, but these will dry out as soon as the basal roots are growing. Figure 35 illustrates this polar character of regeneration in defoliated pieces of stem suspended in moist air. When, however, a long defoliated stem is cut into as many pieces as there are nodes all the dormant nodal buds of the stem will grow out into shoots

(Fig. 36). The stems in Figs. 35 and 36 were all cut out at the same time and suspended simultaneously in moist air in the same aquarium.

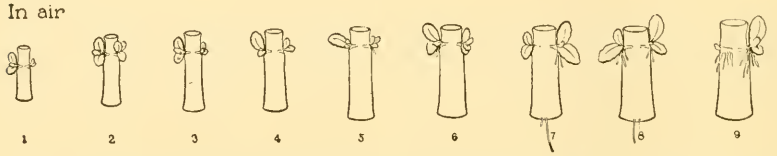

Fig. 36.—Stem cut into 9 small pieces with one node each. Suspended in same aquarium and simultaneously with large stems in Fig. 35. (1) was the most apical, (9) the most basal piece, the serial number denoting the original position of the pieces in the plant. Each piece of stem forms two shoots in its node, but the relative mass of the shoots varies with the relative mass of the stem, not with the serial number of the node.

Fig. 37.—Same experiments as Fig. 35, only that the long piece of stem each was put with its base in water. Duration of experiment from Sept. 27 to Oct. 22. All stems were cut from one plant. Rate of regeneration in proportion to the mass of each piece.

The results remain about the same when the basal ends of the pieces are dipped into water, the only difference being that often

not only the 2 buds in the most apical node of a long piece of stem grow out but also 1 or 2 buds of the node below (Fig. 37). The rate of growth of shoots and roots is also greater in the stems put with their base in water (Fig. 37) than when the stems are suspended in moist air (Fig. 35). When pieces of stem with only 1 node each are put into water each piece forms shoots at its node (Fig. 38).

By comparing the amount of shoots formed simultaneously in the one-node pieces in Fig. 36 or 38 and in the four-node pieces in Fig. 35 or 37 the reader will notice that the shoots are greater in the larger pieces of stem, and the same fact can be observed in

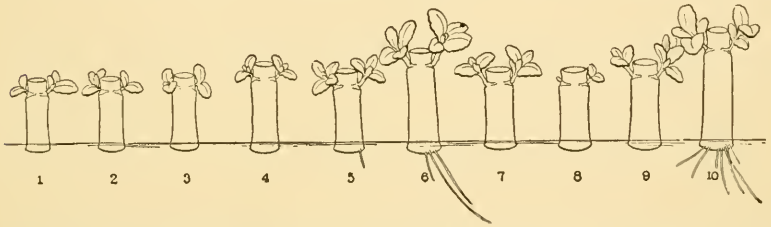

FIG. 38.—Stem of one plant cut into 10 small pieces, the serial number indicating their position in the plant, (1) being the most apical, (10) the most basal piece. Base in water. Experiment simultaneous with that represented in Fig. 37. Each piece has formed 2 shoots, the relative size of which does not follow the serial number of the stem, but the relative mass of the stem. The size of each shoot of the pieces is much smaller than the size of the shoots formed simultaneously by the larger stems in Fig. 37. The latter stems all have roots, while only the two largest pieces of stems (6) and (10) in Fig. 38 have formed them.

all the other figures in this chapter. It is almost obvious from a glance at the figures that the mass of shoots formed increases with the mass of the stem. It is intended to show that within the limits of the experimental errors, the mass of dry weight of shoots produced per gram of dry weight of stem under the same conditions and in the same time is about the same, regardless of whether the stems are long or whether they are subdivided into one-node pieces.

The defoliated stem of a very large plant was cut into 5 pieces, each possessing 4 nodes (Fig. 37), and the defoliated stem of a second plant was cut into 10 small pieces of 1 node each (Fig. 38). The pieces dipped with their base into water and the large and small pieces were suspended in the same aquarium. The experiment lasted from Sept. 27 to Oct. 22, 1922. The shoots were then cut off and both shoots and stems were dried for 24

hours in an oven at about 100°C. The result was as follows: The dry weight of the 5 large stems (Fig. 37) was 13.670 grams, and the dry weight of their 16 shoots was 0.495 gram. The shoot production was therefore 36 milligrams per gram of stem (all measured in dry weight). The dry weight of the 10 short pieces of stem with 1 node each (Fig. 38) was 2.880 grams, and the dry weight of 19 shoots was 0.115 gram, or 1 gram of dry weight of stem produced 40 milligrams of dry weight of leaves. These two figures, 40 milligrams and 36 milligrams, agree sufficiently closely to show that under equal conditions the production of shoots of defoliated pieces of stem occurs in approximate proportion with the mass of the piece of (defoliated) stem; or, in other words, the mass of shoots produced at the apex of the large defoliated stems of Fig. 37 is approximately equal to the mass of shoots the same stems would have produced had all the dormant buds of each stem been able to grow out.

The experiment in Fig. 35, where the stems were suspended entirely in moist air, gave a similar result. The experiment lasted from Oct. 4 to Nov. 7. Five large stems with 4 nodes each (Fig. 35) having a dry weight of 5.486 grams produced 10 shoots with a dry weight of 0.114 gram; *i.e.*, 20.8 milligrams of shoot per gram of stem.

Four short pieces of stem with 2 nodes each, having a dry weight of 3.214 grams, produced 8 shoots with a dry weight of 0.0668 gram; *i.e.*, 20.7 milligrams of shoot per gram of stem.

A third stem was cut into 9 pieces with 1 node each (Fig. 36) possessing a dry weight of 3.270 grams, giving rise to 17 shoots with a dry weight of 0.050 gram; *i.e.*, 15.3 milligrams of shoot per gram of stem.

The first two figures are identical, the last figure is a little low. In these experiments the end of the piece may suffer (by drying out or falling a prey to fungi) and this creates an error which is especially noticeable when a stem is cut into many small pieces. But in spite of these sources of error the results are remarkably clear and consistent.

It seemed of interest to compare the behavior of defoliated stems split longitudinally. In this case the two halves should give approximately equal results.

2. Experiments with Stems Split Longitudinally.—Experiments were made with stems split longitudinally as indicated in Fig. 39. Only pieces from the middle of the stem of a large

plant were used for reasons to be given later. Stems with 4 nodes each, were split longitudinally and one half was cut transversely into 2 pieces with 2 nodes each, a', b', and c', d', respectively (see Fig. 39). The other half with the 4 nodes a, b, c, and d was not cut. All 3 pieces were put with their base into water. It was to be expected that the sum of the dry weight of the shoots produced by the 2 small pieces with 2 nodes each should

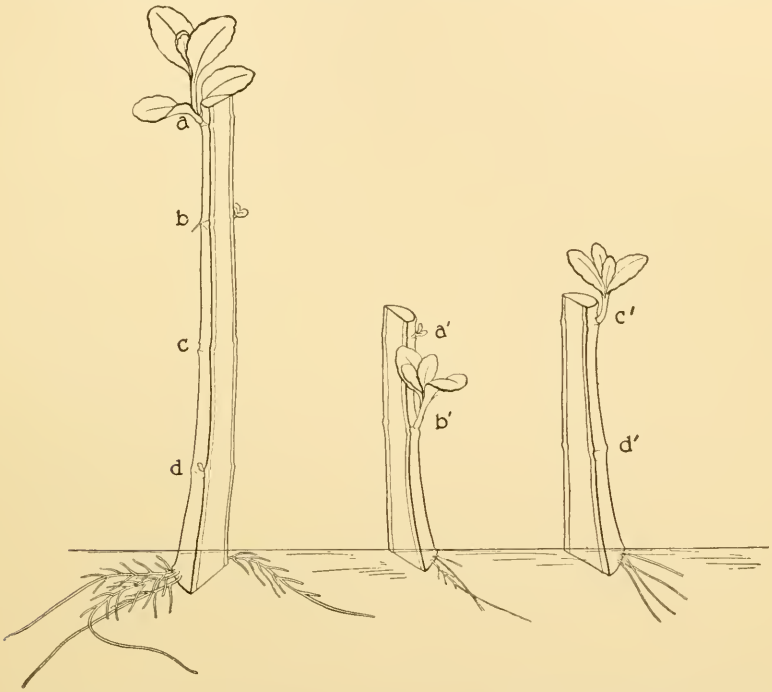

FIG. 39.—Piece of stem with 4 nodes, a, b, c, d, split longitudinally. One-half cut transversely into two pieces, a', b', and c', d'. The half a, b, c, d, produces 1 shoot which about equals in mass the 2 shoots produced by a', b', and c', d'. Duration of experiment, Dec. 9, 1921 to Jan. 4, 1922.

equal the dry weight of the shoots produced by the larger pieces with 4 nodes each. Figure 39 shows at a glance that this is approximately the case and the dry weight determinations confirm this.

The first experiment was carried out on 7 stems, a second experiment on 16 stems. Table XV gives the result. The four-node pieces produced 36 milligrams dry weight of shoots per gram dry weight of stem; while the two-node pieces produced

38.7 milligrams of shoots per gram of stem, practically identical results.

It is therefore obvious that the dry weight of the sum of the shoots produced by the small pieces a', b', and c', d', approximately equals the dry weight of the shoots produced by the big pieces, a, b, c, and d (Fig. 39), or, in other words, the mass of shoot produced at the apex of the large pieces is approximately equal to the dry weight of the shoots the same stems would have produced had the buds of every second node been able to grow.

TABLE XV

Experiment No.	Duration of experiment	Number of pieces	Dry weight of shoots produced, grams	Dry weight of stems, grams	Shoots produced per gram of stem, milligrams
I	1921 Nov. 3–Dec. 6.	7 four-node pieces, a, b, c, d.	0.1545	4.290	36.0
		14 small pieces, a', b', c', d'.	0.147	3.822	38.7
II	1921–22 Dec. 8–Jan. 10.	16 four-node pieces, a, b, c, d.	0.750	16.646	45.0
		32 two-node pieces, a', b', c', d'.	0.577	14.527	39.5

3. Further Experiments on Small and Large Pieces of the Same Stem.—A third series of experiments was as follows: Long pieces of stem, containing about 10 nodes, were cut out from the same plant which was more than 1 year old (Fig. 40). The middle piece of about 6 nodes (piece 3 in Fig. 40) served for the experiment, and two small pieces, 4 and 5, containing 1 node each, situated basally from the large middle piece in the same stem, serving as controls. In other experiments of the same character pieces containing about 14 nodes were cut out from the stem of the same plant; 2 small pieces at the base, each containing 2 nodes (4 and 5, Fig. 41), were used as controls, while the middle piece (3, Fig. 41) served for the main experiment. All the pieces dipped with their base into water.

It is obvious from Figs. 40 and 41 that the large pieces of stem (3) produced larger masses of shoots than the small pieces 1 and

2 or 4 and 5 during the same time and under equal conditions. It may also be pointed out that these large middle pieces (3) formed their basal roots earlier than the small pieces and that the mass of their roots remained greater than the mass of roots in the small pieces (Figs. 40 and 41).

FIG. 40.—Five pieces cut from the stem of the same plant, (1) and (2) apical pieces with 1 node each, (3) middle piece with 6 nodes, and (4) and (5) the basal pieces with 1 node each. The large middle piece produces larger shoots than either the more apical or more basal small pieces. The large middle piece has ample roots while only the longer basal piece commences to form a root. Duration of experiment, Oct. 25 to Nov. 21, 1921.

It turned out that the shoot production in the most apical pieces of stems 1 and 2 was usually irregular, as a rule too small, so that these pieces were not well usable as controls. The basal pieces 4 and 5, however, behaved normally. It seems that this abnormal behavior of the small apical pieces is found as long as the leaves connected with this piece are still small and growing, and is perhaps due to the fact that the bud from which later

the regeneration of shoots in the stem proceeds is not yet perfectly developed. It is well, therefore, not to use in these experiments that part of the stem which is too near the apex. It may also be well not to use pieces of stem too near the roots. After 3 to 5 weeks the dry weight of the shoots and of the stem

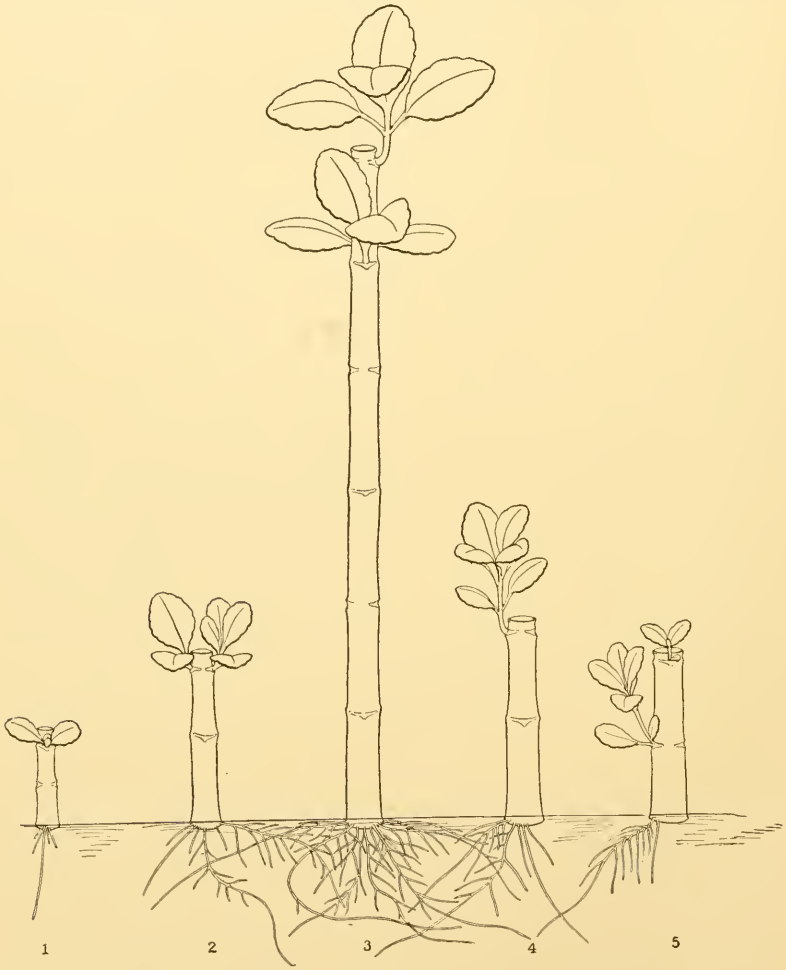

FIG. 41.—Similar experiment as Fig. 40, except that the small pieces have 2 nodes each. Shoots and roots are formed in proportion to mass of stem. Duration of experiment, Nov. 16 to Dec. 19, 1921.

used in these experiments was determined. Since some of the small pieces of stem often fall a victim to fungi only one of the two small basal pieces was used as a control.

EXPERIMENT I.—OCT. 25, 1921, TO NOV. 25, 1921

	GRAMS	DRY WEIGHT OF SHOOTS PER GRAM OF STEM, MILLIGRAMS
Six long pieces with six nodes each.		
Dry weight of stems	9.260	
Dry weight of 13 shoots	0.260	28.0
Dry weight of roots	0.057	
Control. Seven short basal pieces with one node each.		
Dry weight of stems	2.895	
Dry weight of 13 shoots	0.088	30.4
Dry weight of roots	0.003	

It is apparent that the basal control pieces produced approximately the same amount of shoots per gram of stem, namely 30.4 milligrams as compared with 28.0 for the large pieces.

EXPERIMENT II.—NOV. 2, 1921, TO DEC. 6, 1921

	GRAMS	DRY WEIGHT OF SHOOTS PER GRAM OF STEM, MILLIGRAMS
Five long pieces of stem with six nodes each.		
Dry weight of stems	6.486	
Dry weight of 10 shoots	0.272	42.0
Dry weight of roots	0.0458	
Control. Four short basal pieces with one node each.		
Dry weight of stems	1.058	
Dry weight of 8 shoots	0.041	39.0
Dry weight of roots	0.0034	

Again the short basal control pieces produce about as much shoot material per gram (39 milligrams), as the large pieces (43 milligrams).

EXPERIMENT III.—NOV. 16, 1921, TO DEC. 20, 1921

	GRAMS	DRY WEIGHT OF SHOOTS PER GRAM OF STEM, MILLIGRAMS
Nine long pieces of stem with six nodes each.		
Dry weight of stems	18.658	
Dry weight of 26 shoots	0.944	50.3
Dry weight of roots	0.1428	
Control. Eighteen small basal pieces of two nodes each.		
Dry weight of stems	18.147	
Dry weight of 36 shoots	0.800	44.0
Dry weight of roots	0.136	

Experiment IV.—Oct. 22, 1921, to Nov. 15, 1921

	Grams	Dry Weight of Shoots per Gram of Stem, Milligrams
Four long pieces of stem with four nodes each.		
Dry weight of stems	4.214	21.0
Dry weight of 8 shoots	0.089	
Control. Four short basal pieces of two nodes each.		
Dry weight of stems	2.492	19.0
Dry weight of 8 shoots	0.0475	

Experiment V.—Oct. 11, 1921, to Nov. 1, 1921

	Grams	Dry Weight of Shoots per Gram oe Stem, Milligrams
Four long apical stems with six nodes each.		
Dry weight of stems	3.921	
Dry weight of 8 shoots	0.113	29.0
Dry weight of roots	0.0134	
Control. Four basal pieces of two nodes each.		
Dry weight of stems	3.744	24.0
Dry weight of 10 shoots	0.090	

Experiment VI.—Dec. 11, 1921, to Jan. 17, 1922

	Grams	Dry Weight of Shoots per Gram of Stem, Milligrams
Seven long apical stems with six nodes each.		
Dry weight of stems	6.634	
Dry weight of 12 shoots	0.340	51.0
Dry weight of roots	0.0512	
Control. Seven short basal pieces of two nodes each.		
Dry weight of stems	3.560	
Dry weight of 12 shoots	0.1770	49.6
Dry weight of roots	0.0128	

We notice that the differences of shoots produced per gram of dry weight of the controls differ comparatively little from those produced by the large pieces of stem and in some cases the difference is only about 6 per cent. Considering the limitations in the experimental conditions—the fact that part of the stem may not function normally, especially the ends near the cut, or the fact

that individual buds may have been injured by parasites, etc.—the agreement of the figures seems remarkable.

These results leave no doubt that within the limits of accuracy of these experiments the dry weight of the shoots produced at the apex of a long piece of defoliated stem is about equal the mass of shoots the same stem would have produced had the buds in all of its nodes been able to develop.

4. Regeneration of Roots.—The regeneration of the basal roots differs from the formation of apical shoots in this, that the apical shoots begin to grow out almost immediately after the defoliated piece of stem is isolated, while there is a long latent period before the basal roots make their appearance. For this reason quantitative measurements correlating the mass of the basal root formation with the mass of stem require probably a longer time than that selected for our experiments. A glance at the drawings will, however, convince the reader that the root formation commences sooner in the stems with larger mass than in the stems with smaller mass, regardless of the original position of the piece of stem in the plant. Thus in Fig. 40 the large middle piece (3) forms roots before either of the two more basal pieces, and Fig. 41 shows that the relative mass of roots produced seems also to run parallel with the relative mass of the piece. The same phenomenon is shown if we compare Fig. 37 with Fig. 38. It is also obvious in Fig. 39, so that we can say that the mass of roots produced by pieces of defoliated stem of *Bryophyllum calycinum* increases under equal conditions with the mass of the stem.

5. Influence of Light on Regeneration in a Defoliated Stem.—To prove finally that we are dealing with the mass of material produced by assimilation it is necessary to examine the effect of light on regeneration in a defoliated stem. Eight such stems were suspended in an aquarium kept dark by a double cover of black cardboard, and eight equally long defoliated stems were put at the same time into an aquarium exposed in the usual way to daylight. The base of the stems dipped into water. All conditions were alike except the illumination. After 23 days all the stems exposed to light had formed large basal roots and large shoots at the apex (right stem in Fig. 42). At the same time none of the stems in the dark had formed a single basal root though some had formed tiny air roots (left stem in Fig. 42). The shoots formed in the dark had a small mass and the typical etiolated shape. The most striking phenomenon was the lack

FIG. 42.—Influence of light on root and shoot formation of stem. In the dark no roots are formed; in light ample roots are formed. The mass of shoots formed in the dark is small compared with the mass of shoots formed in light.

of root formation at the base of the stem in the dark. We shall see later that the favorable influence of the leaf on root formation in the stem also disappears when the leaf is deprived of light.

These experiments indicate that the quantity of regeneration in a defoliated piece of stem depends upon the mass of assimilation products in the stem. This is rendered possible by the fact that the cortex of the stem contains chlorophyll.

6. Child's Hypothesis of Axial Gradients.—We may, in passing, discuss here a hypothesis of the polar character of regeneration which Child has defended in a large number of publications, namely that the polar character of regeneration is due to an axial gradient of metabolism, where the rate of metabolism is supposed to be a maximum at one pole of an organ or an animal and a minimum at the opposite pole, declining steadily with the distance of the piece from the pole.[1] Since we have shown that both roots and shoots grow in proportion to the mass of the piece of stem there should be two opposite gradients of metabolism in the stem, one increasing from apex to base to explain root formation, and the other from base to apex to explain shoot formation. Child measures the relative metabolism of a piece by the time required to dissolve the piece in a solution of potassium cyanide. The unit for the measurement of metabolism is the calory and the calories produced by an animal or plant or by one of its segments are not measured by the time required to dissolve the animal or plant in a solution of potassium cyanide. Furthermore, regeneration is due to synthetic processes and the only approximate measure for this at present is the dry weight of the organs produced. If the polar character of regeneration were due to a synthetic gradient existing in the stem it would follow that the rate of synthesis or growth of a bud should be the greater the nearer the bud is to the apex of a plant. If we, therefore, cut a long piece of stem into as many pieces as there are nodes, then shoot formation should not start in the different pieces of stem simultaneously but successively in the order in which the pieces were originally arranged in the stem, beginning first in the most apical and last in the most basal piece; and the rate of growth of the bud of each piece should follow the same serial order. Figures 36 and 38 give the results of exactly such experiments. The pieces designated as 1 to 9 (Fig. 36) were cut out from one stem and so were the pieces 1 to 10 (Fig. 38). The piece desig-

[1] Child, C. M.: "Senescence and Rejuvenescence," Chicago, 1915.

nated in the two figures as 1 was nearest the apex, and the serial number denotes the order of the original position of each piece in the stem. The growth of shoots did not commence first in the most apical piece 1, next in the piece marked 2, etc., but growth started practically simultaneously in all pieces; if there was a lag in the time, it was usually in the most apical pieces and for the reason already given. Furthermore, the rate of growth of the shoots did not follow the serial order but was clearly in proportion to the mass of the piece of stem, as a glance at the figures shows. Child's assumption is contrary to the facts, not only so far as the regeneration of shoots but also so far as that of roots is concerned.

CHAPTER VIII

THE ROLE OF THE INHIBITING EFFECT OF RAPIDLY GROWING PARTS IN THE REGENERATION OF THE STEM

The preceding chapter has shown that the mass relation determines the quantity of regeneration in a piece of defoliated stem. When roots grow only at the basal and shoots only at the apical end of the piece it follows that all the material available for shoot and root formation in the stem must collect at the ends of the piece; since only in this way can it happen that the total amount of shoot formation at the apex of a long piece of stem is in direct proportion to the mass of the stem.

The polar character of the regeneration is in a stem of *Bryophyllum* not the direct and primary phenomenon which it is generally assumed to be, but is more complicated. Roots and shoots can appear in all nodes of a piece of stem and the fact that finally roots are left only at the base and shoots only at the apex is due to a secondary effect; namely, to the fact that the more rapidly growing shoots or roots inhibit the growth of the more slowly growing shoots or roots. This phenomenon of inhibition has already been described for the leaf where the rapid growth of shoots in one part of the leaf causes the flow of all the sap of the leaf to that part, thereby inhibiting the growth in the other notches.

Stems with two leaves at the base were suspended vertically in moist air (Fig. 43). Within 5 days roots and shoots commenced to form, but the root formation was not confined to the base appeared in all the nodes (1 to 4, Fig. 43), with the exception of the most apical node; and shoots began to form not only in the most apical node 0 but also in the node 1 below. There is little suggestion of a polar character of the regeneration in Fig. 43. Six days later (Fig. 44) the most apical shoots had grown more rapidly than the shoots in the nodes below, the growth of which was now completely inhibited. Furthermore, roots began to grow at the extreme base of the stem. These latter roots grew more rapidly and in greater numbers than the other

roots, thereby inhibiting the further growth of other roots in nodes 1, 2, 3, and 4. These latter roots soon wilted and fell off.

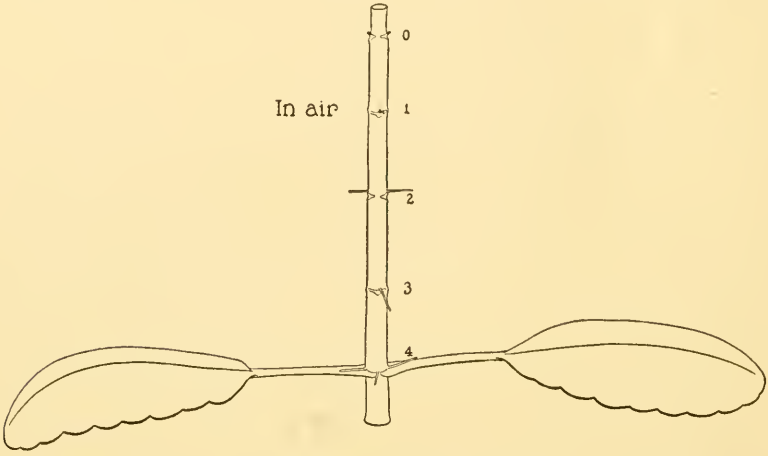

FIG. 43.—The polar character of regeneration in the stem is not pronounced in the beginning. Roots form at first in nodes 1, 2, 3, and 4, and shoots in the most apical node 0, and the node below. Stem suspended in moist air. Oct. 18 to 23.

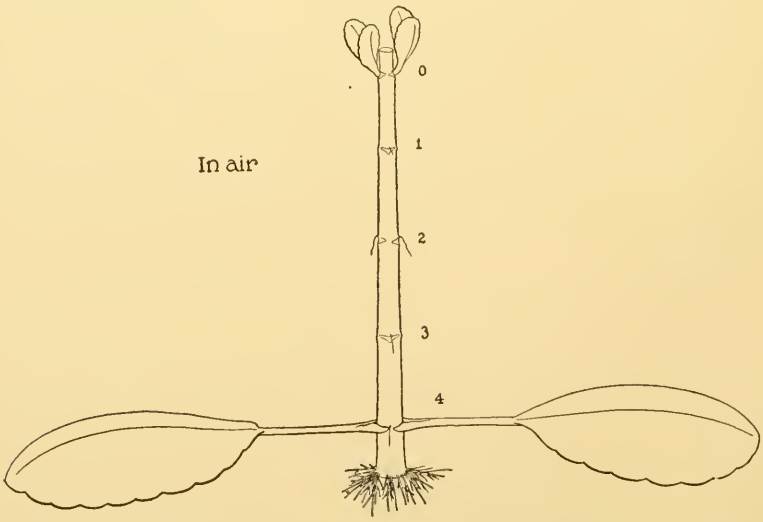

FIG. 44.—The same stem as in Fig. 43 six days later (Oct. 29). The most apicol node has grown vigorously, ehereby suppressing the growth of shoots in node 1 below. Roots appear at the base.

Figure 45 shows the powerful development of roots at the extreme base and of shoots at the extreme apex, and the remnants of the

wilted roots in the upper nodes. A few days later, it was difficult to find traces of these latter roots. The polar character of the regeneration as it appears ultimately is therefore a secondary phenomenon.

In a completely defoliated stem the polar character of regeneration is also less pronounced at the beginning. Figure 46 shows the appearance of a defoliated stem after 13 days. In order to accelerate regeneration the base of the stem was put into water. Roots had formed at first in nodes 2, 3, 4, and 5. A little later roots appeared at the extreme base dipping into

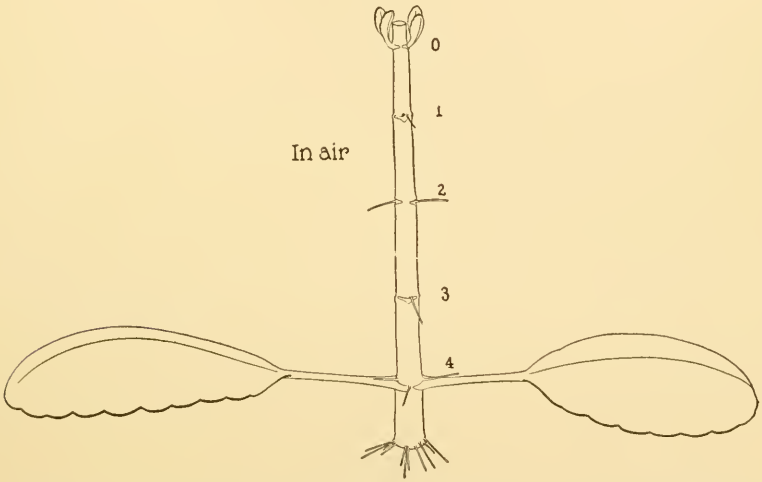

FIG. 45.—The same stem as in Figs. 43 and 44 on Nov. 7. The roots at the base have grown vigorously, suppressing the further growth of roots in nodes 1, 2, 3, and 4. These latter roots are now wilted and about to fall off. The stem has now a shoot only at the apex and roots only at the base, thus exhibiting the polar character of regeneration which did not exist in the earlier stages of regeneration.

water, and now the roots in the higher nodes began to wilt. The stem is drawn in that stage (on the thirteenth day). Later the wilted roots fell off. Shoots had formed not only in the most apical node marked 0 but also in the first node below; the more rapid growth of the shoots in the most apical node inhibited the growth of the shoots in the node below.

The phenomena here described are not exceptions but the rule. The polar character of the regeneration is not obvious from the beginning, but is a secondary phenomenon and the explanation

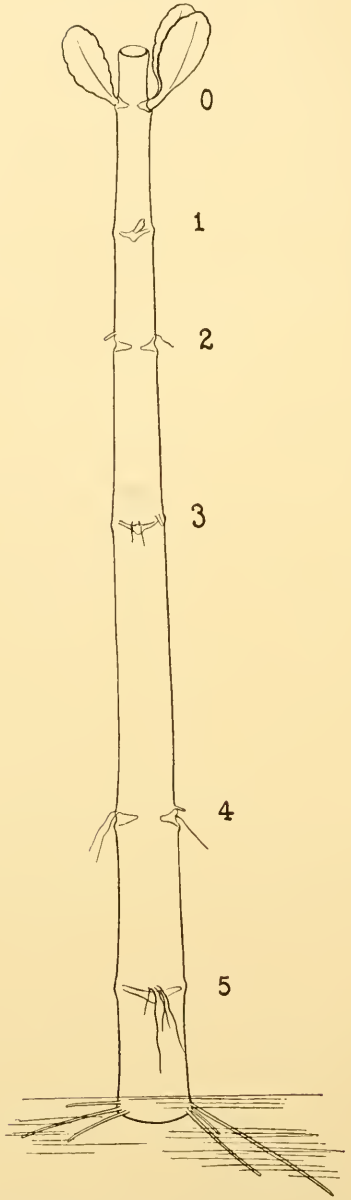

FIG. 46.—Stem suspended with base in water. Oct. 16 to 29. Roots had originally formed in nodes 1, 2, 3, 4, and 5; these roots wilted and ceased to grow when the basal roots commenced to grow.

seems to be as follows. There is, as a rule, enough sap present in a piece of stem at the beginning to permit the starting of root formation in many nodes, and of shoot formation in more nodes than the most apical ones. More sap, however, will collect at the two ends of the stem than in the middle and this causes a slight acceleration of root formation at the base and of shoot formation at the extreme apical node. This causes as a secondary result a vigorous flow of the sap from the whole stem

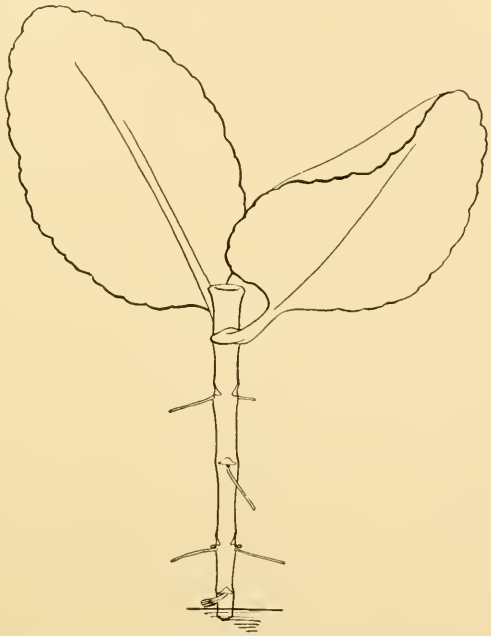

FIG. 47.—Proof that the polar character of regeneration in a stem suspended vertically is independent of gravity. Stem in Fig. 47 inverted, with apex dipping into water and base above. Shoots commence to appear at the three most apical nodes, and roots in the intermediate nodes between the most apical and basal one. Mar. 4 to 12, 1919.

to these two ends whereby the growth in the intermediate nodes is inhibited. The organs which commenced to grow in the intermediate nodes now stop growing or wilt.

The question presented itself to what extent this result is determined by gravity. In Figs. 43 to 45 the stems were in a vertical upright position. It was found that the result was the same when stems were suspended in moist air in an inverted position, the apex being below and the base above. Figures 47

and 48 give the result of such an experiment. The apex of the stem in this case was dipped into water, but this is not necessary. Figure 47 shows the result after 8 days. Three nodes had formed roots and the three most apical nodes had commenced to form shoots. Ten days later (Fig. 48) only the most apical shoots had continued to grow, thereby suppressing the shoots that had commenced to grow in the other nodes. The roots at the extreme base of the stem had now commenced to grow and the other roots had wilted. In Figs. 47 and 48 everything happened in the

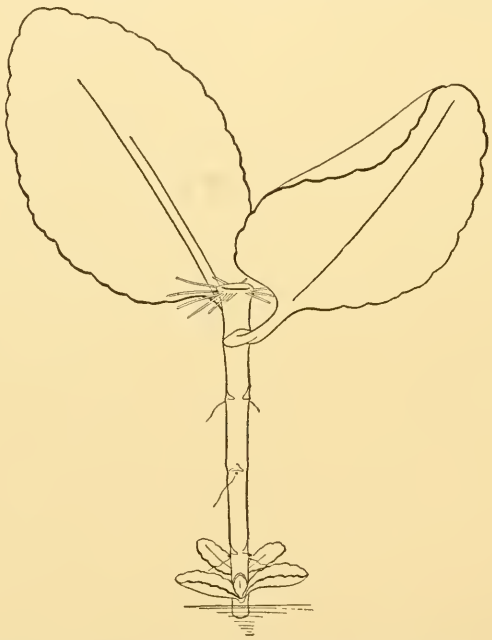

Fig. 48.—Same stem as in Fig. 47 10 days later. Roots have formed at the base and shoots in apex, just as in stems suspended in upright position.

inverted position of the stem as it did in the upright position in Figs. 43 to 45, and gravity has therefore little if anything to do with the polar character of regeneration in a stem of *Bryophyllum calycinum* in vertical position. We shall see later that it is different when the stem is suspended horizontally.

The polar character of regeneration can be obliterated when the stem is put partly under water. It is in this case necessary that one of the two ends of the stem should be in air, since otherwise regeneration may fail to occur. When a whole defoliated

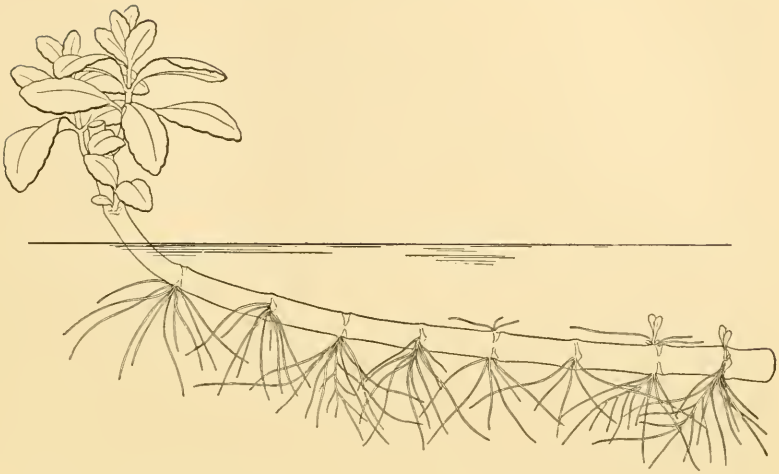

FIG. 49.—Stem suspended in water with the exception of the apex. Roots now permanent in all the nodes in water. Polarity as regards root formation disappears. Oct. 12, 1920 to Jan. 4, 1921.

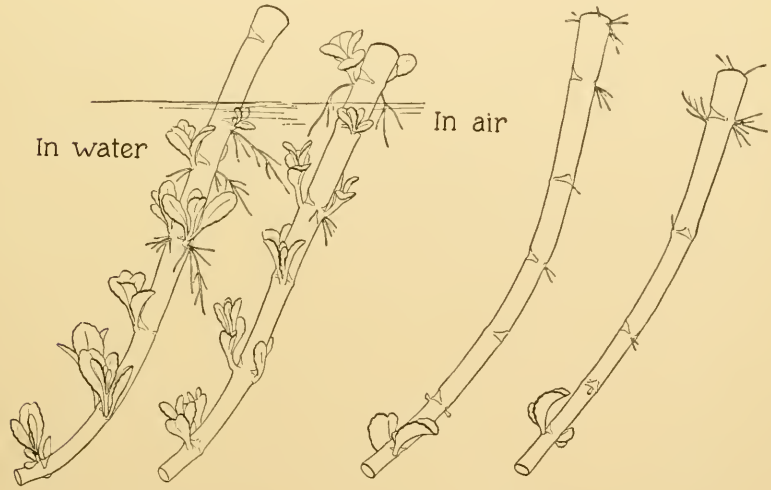

FIG. 50. FIG. 51.

FIG. 50.—When stems are put with their apices into water, all the nodes can form shoots. Polarity disappears. Oct. 7 to 30, 1919.

FIG. 51.—Stems suspended simultaneously in air form shoots only at apex. Oct. 7 to 30, 1919.

stem (with the exception of the apical end) is in water the roots form in all the nodes and do not wilt since they have enough water (Fig. 49). Shoots formed in this case only at the apex. When the whole stem is in water, with the exception of the basal end, shoots may be formed in all the nodes (Fig. 50) especially in old stems. Stems suspended in moist air in the same position form shoots only at the apical end (Fig. 51). It is obvious that the limited amount of water available in a stem suspended in moist air (even if it dips with one end into water) is a contributory cause to the polar character of regeneration in the stem, since when the stem is submersed in water the formation of roots and shoots is not confined to the two poles of the stem.

It may be stated incidentally that in the leaves formed on a stem under water the ratio of width to length is smaller than in leaves formed in air.

PART II
POLARITY IN REGENERATION

CHAPTER IX

THE INFLUENCE OF THE LEAF ON THE REGENERATION IN THE STEM

Not every case of regeneration is polar in character. The healing of a wound is not a polar phenomenon; nor is the formation of roots and shoots in an isolated leaf of *Bryophyllum*. The final distribution of roots and shoots in a defoliated piece of stem of *Bryophyllum* suspended vertically and upright in moist air is, however, a pronounced example of the polar form of regeneration. It is our purpose in this second part of the book to find out to what extent the mass relation can aid us in the investigation of the cause of polar character of regeneration in the stem of this plant.

There is little doubt that the formation of roots at the base and of shoots at the apex of a defoliated piece of stem is determined by the descending and ascending sap respectively, since the quantity of both root and shoot formation increases with the mass of the stem, provided that the stem is exposed to light.

The question is then: What determines the difference in the action of the descending and ascending sap? There are, as far as the writer can see, two possibilities. The polar character of the regeneration in the stem is either due to chemical differences in the ascending and descending sap; or the polar character of the regeneration is due to the fact that the roots and shoots do not originate from the same type of cells or anlagen and that the cells or anlagen for root formation are reached exclusively or preferably by the descending sap, while the anlagen for shoot formation (two of which exist in each node) are reached exclusively or preferably by the ascending sap. Either of these two assumptions might explain the polar character of regeneration in the stem of *Bryophyllum* from the point of view of the mass relation.

There can be no doubt that the ascending and descending sap in a normal plant growing in the soil are chemically different. The root takes up the nitrogen from the soil in the form of KNO_3 or NH_3 and KNO_3 is reduced to the nitrite in the root or

in the stem. Schimper has shown that the nitrites are present in the leaf when the plant is kept in the dark, but that they disappear in the light, provided that the leaf contains normal, non-etiolated chlorophyll. Baudisch[1] found that when an aqueous solution of potassium nitrite and methyl alcohol is exposed to ultraviolet light, formhydroxamic acid is formed. Baly, Heilbron, and Hudson[2] have found that solutions of nitrite or nitrate containing formaldehyde also form formhydroxamic acid in the light. It is possible that from the formhydroxamic acid amino-acids may be formed as suggested by Baudisch as well as by Baly, Heilbron, and Hudson. The nitrites are therefore carried in the ascending sap. On the other hand, the sugars are synthetized in the presence of light from CO_2 in the leaves containing chlorophyll, and the sugars required for the synthesis of the material for roots must be carried by the descending sap. There exists then unquestionably a difference in the chemical nature of the ascending and descending sap in a normal plant exposed to light. The question is whether this and other possible differences (*e.g.* of Ph) are the cause of the polar character of regeneration in the stem or whether the polarity exists regardless of such differences. To test this it is necessary to study the influence of the leaf on the polar character of regeneration, since the sap in the leaf contains all the substances (specific and non-specific) required for the growth of both roots and shoots. This is no mere surmise, but is demonstrated by the fact that from each notch of an isolated leaf both roots and shoots can arise, and that the mass of shoots and roots produced varies in proportion with the mass of the leaf. This leaves no doubt that the same tissue sap can give rise to entirely different organs and that the cause of the difference must lie in differences in the chemical and physical constitution of the cells from which the different organs arise. It will be shown in this chapter that the shoots or roots produced in a small isolated piece of stem containing one or more leaves originate almost entirely from the sap sent out by the leaf. If it is intended to explain the polar character of the regeneration in this case on the basis of a chemical difference of the sap sent out by the leaf to the two opposite poles of the stem it will be necessary first to

[1] Baudisch, O.: *Ber. d. d. chem. Ges.*, vol. xliv, p. 1009, 1911.

[2] Baly, E. C., Heilbron, I. M., and Hudson, D. P.: *Trans. Chem. Soc.*, vol. cxxi, p. 1078, 1922.

explain how the sap sent out by a leaf can be separated into two chemically distinct masses, one to travel in an ascending the other in a descending direction in the stem. The writer is not aware that such an assumption is justified on the basis of our present knowledge.

Slender stems with 5 nodes (designated as 0, 1, 2, 3, and 4) were cut out from plants almost but not quite one year old. All the leaves were removed except the pair of leaves in the third node (Fig. 52). The stems were split longitudinally between the two leaves, one leaf being left in connection with its half stem (I, Fig. 52), while the other leaf was detached and suspended with its apex dipping into water (III, Fig. 52). The two half stems I and II dipped also with their base into water. Half stem I, with a leaf attached in node 2, produced larger shoots at the apex than the other half of the stem II with no leaf. The difference in root formation was in the same sense and still greater. The experiment lasted from Oct. 18 to Nov. 9. Six half stems without leaves (II, Fig. 52) produced 28 milligrams dry weight of shoots, while the other six half stems each with a leaf produced (during the same time under equal conditions) 237 milligrams dry weight of shoots. Of this quantity about 209 milligrams were therefore furnished by the leaves. The 6 isolated sister leaves (III, Fig. 52) produced 292 milligrams of shoots. Hence more than two-thirds of the material available for shoot formation in the leaf was used for shoot formation in the apex of the stem. The 6 isolated leaves produced 102 milligrams dry weight of roots while the 6 half stems with leaves attached produced 136 milligrams. Hence the total quantity of regeneration in the stems was 373 milligrams, while the total quantity of regeneration in the isolated leaves was 394 milligrams. This shows that the regeneration in the stems was determined chiefly by the sap sent out by the leaves; the leaves in connection with the stem produced neither roots nor shoots.

The pieces of half stem without leaf had just commenced to produce a few roots. It may be well to make use of this fact to point out that the influence of the leaf on the root formation in the stem cannot be ascribed merely to water being sent by the leaf into the stem, since in this experiment stems both with and without leaf (I and II, Fig. 52) were each placed with their base in water, so that the stems without leaves had all the water they needed for root formation at the base; yet their root formation

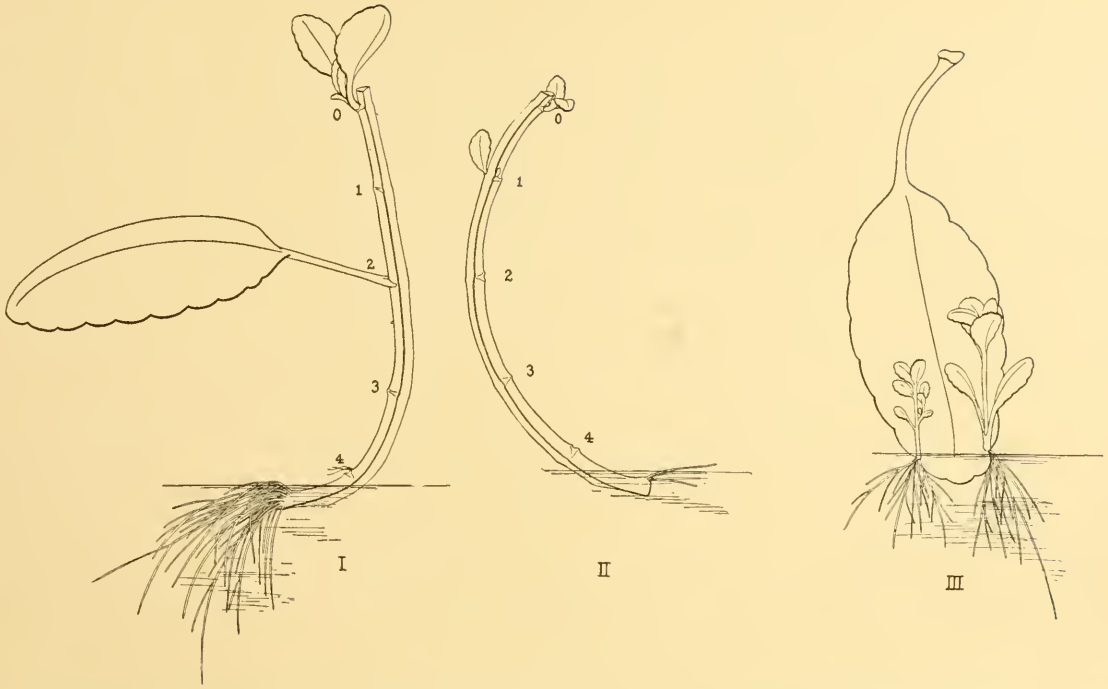

FIG. 52.—Stem with 5 nodes and a pair of leaves in the middle (node 2) split longitudinally: I, half with leaf attached, forms powerful roots at base, and shoots at apex; II, other half of stem, without leaf, forms smaller shoots and fewer roots; III, leaf detached from II, forms shoots at apex. Base of stems and apex of leaf III dipping into water. Oct. 18 to Nov. 9, 1923.

was delayed and limited in comparison with that of a stem possessing a leaf. The leaf furnished, therefore, something more than water for the formation of the roots. The same reasoning holds for the influence of the leaf on the formation of shoots in the stem, since the stem dipping into water could absorb all the water needed for shoot formation.

The total results of the experiment are given in Table XVI.

TABLE XVI

	Dry weight of leaves, grams	Dry weight of stems, grams	Dry weight of regenerated	
			Shoots, grams	Roots, grams
I. Six half stems with leaves.....	1.993	1.889	0.237	0.136
II. Six half stems without leaves..	.....	1.836	0.028	
III. Six leaves.....	2.005	.....	0.292	0.102

Figure 52 shows incidentally that in this case the leaf left in connection with the half stem did not produce any shoots or roots. This is a confirmation of the statement made in an earlier chapter that if the piece of stem to which a leaf is attached is sufficiently large and if the leaf is in air the formation of shoots and roots in the leaf is generally completely suppressed. This will be supported by a large number of the experiments which will be described in the remaining chapters of the book, wherever the piece of stem connected with the leaf is large and the leaf is in air.

In this experiment the regeneration of roots was completely due to the material sent out by the leaf into the stem and over two-thirds of the dry weight of the shoots regenerated by the stem was furnished by the sap from the leaf. Yet the character of the regeneration in the stem was as clearly polar as in the completely defoliated stems described in a preceding chapter.

It was to be expected that the quantity of regeneration in an isolated piece of stem should vary with the mass of the leaf attached. In 8 stems, of 5 nodes each, all the leaves except those in the third node were removed and the stems were split lengthwise. One leaf remained intact, while the sister leaf was reduced by cutting away about two-thirds. The stems were suspended so that their base dipped into water. Figure 53 shows that the half stems with reduced leaves produce a correspondingly smaller mass of apical shoots and roots. Table XVII gives the results.

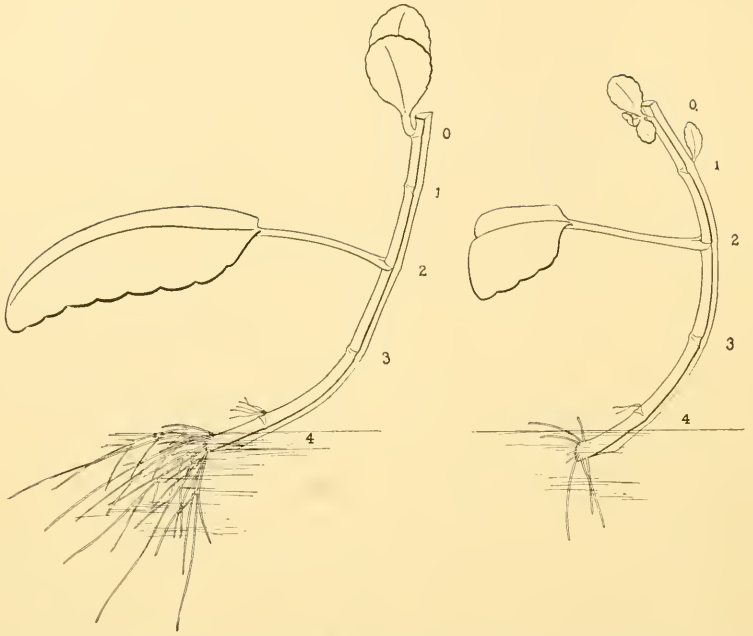

FIG. 53.—Stem split longitudinally, each half with leaf in middle. Left, whole leaf; right, more than half of leaf cut off. Root and shoot production in the two pieces of stem increases with mass of leaf.

TABLE XVII

	Dry weight of leaves, grams	Dry weight of stems, grams	Dry weight of regenerated	
			Shoots, grams	Roots, grams
Eight half stems with whole leaf...	3.550	3.318	0.404	0.218
Eight half stems with reduced leaf..	1.273	3.503	0.201	0.046

If account is taken of the fact that part of the material sent by the leaf into the stem is consumed for the growth of the latter (as was shown in Chapter VI), the mass of shoots produced in the stem varies approximately with the mass of the leaf.

In these experiments the base of the stem dipped into water and it might be argued that the substances taken by the stem therefrom and carried in the ascending sap might have had a share in this result. For this reason the experiments were repeated on stems suspended entirely in moist air. The result was essentially the same.

CHAPTER X

THE INFLUENCE OF GRAVITY ON THE POLAR CHARACTER OF REGENERATION IN A STEM OF *BRYOPHYLLUM*

1. Introductory Remarks.—The decision between the two possibilities for the explanation of the polar character of regeneration in the stem can be given with the aid of gravity. It had been suggested in a preceding chapter that we must distinguish in a plant between the sap which flows in regular vessels and the tissue sap which exists in the tissue spaces. This latter sap can follow the influence of gravity when the tissue spaces are sufficiently large; while the sap in the vessels cannot follow gravity at all or only to a negligible extent. We shall see a corroboration of this suggestion later in this chapter. The fact that the tissue sap follows gravity gives us a chance to decide whether the polar character of regeneration in the stem is due to any chemical differences between ascending and descending sap or whether it is due to any difference in the nature of the tissues which these two kinds of sap reach primarily in the plant; the ascending sap reaching primarily the anlagen for shoots while the descending sap reaches primarily the tissues which give rise to roots. We shall see that in a stem suspended horizontally the descending sap from a leaf collects in the lower side of such a stem, giving rise to the formation of roots; and that this formation of roots on the under side of a stem increases with the mass of an apical leaf. If now the polar character of regeneration in a stem were due to a chemical difference between the ascending and descending sap from a leaf, such a root formation should not occur on the under side of the apical part of a stem which has a leaf at its base, since, in this case, the ascending sap should contain shoot-forming but no root-forming substances. We shall see, however, that a leaf at the base of a stem causes also root formation on the under side of the apical part of a stem placed horizontally, and that this root formation is exclusively due to the ascending sap sent out by the basal leaf. This leaves little doubt that the influence of a

leaf on the polar character of the regeneration in a vertical stem of *Bryophyllum* is due not to any difference in the chemical constitution of the ascending and descending sap from a leaf, but is due to the fact that the descending sap in a stem suspended vertically reaches primarily the anlagen for root formation while the ascending sap reaches primarily the anlagen for shoot formation. Before going into the details of these experiments it will be necessary to discuss some other effect of gravity on the stem; namely, the geotropic curvature of a stem of *Bryophyllum* placed in a horizontal position. When stems of *Bryophyllum* are placed in a horizontal position and kept in moist air, the originally straight stem undergoes a curvature whereby the lower side of the stem becomes convex and the upper concave (Fig. 54, *a*). The rate of this curvature increases with the mass of the leaf. In order to demonstrate this fact a few remarks about the mechanism of this curvature and the method of its investigation may be desirable.

2. Influence of the Leaf on Geotropic Curvature.—In these experiments it is necessary to remember that the geotropic curvature of a stem is the resultant of two opposing forces. One is, as we shall see, the excess of longitudinal growth of cortical tissue on the lower side of the stem suspended horizontally over that of the rest of the stem; the opposing force is the rigidity of the upper layers of the stem, chiefly the wood. When the wood is too hard the stem cannot bend. The influence of equal masses of an apical leaf on the rate of geotropic curvature of two stems can only be equal if the rigidity of the wood is identical in the two stems, a condition which cannot often be fulfilled. Quantitative work of this kind must therefore be statistical; but it is only intended here to prove in a semi-quantitative way that the rate of geotropic curvature of a stem increases with the mass of the apical leaf. It is necessary to select for experimentation only the more apical parts of the stems of young plants where the wood is still soft or not too rigid, otherwise little or no curvature is possible. The method of the experiments is illustrated in Fig. 54. Stems of about equal flexibility were selected and defoliated with the exception of one leaf at the apex. With the aid of a string around the petiole of the leaf, the stem was suspended in moist air. In order to secure a horizontal position of the stem, the latter was put on a wire netting bent in the shape of a U, on the bottom of which the stem was allowed to rest (Fig. 54). The meshes

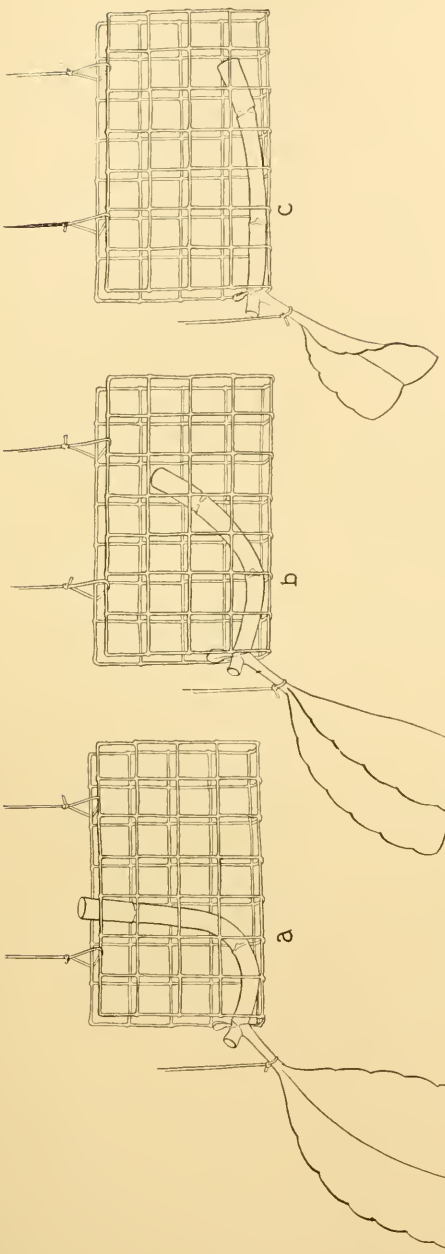

FIG. 54.—Influence of mass of leaf on rate of curvature, *a*, whole leaf; *b*, half leaf; *c*, about one-fourth of a leaf. Curvature is greatest in *a*, least in *c*. Apr. 27 to May 8.

of the net were squares with a side of ½ inch and the squares of the wire netting made it easy to follow and measure the slope of geotropic curvature from day to day. It was found that under proper conditions this curvature increases with the mass of the leaf as intimated in Fig. 54. Three stems (*a*, *b*, *c*) were selected, each having a leaf at the apex. The leaves in *b* and *c* were partly cut off so that the mass of the leaves in *a*, *b*, and *c* was roughly in the ratio of 1:½:¼. The drawing was made after 11 days. The figure shows that the degree of geotropic curvature in the

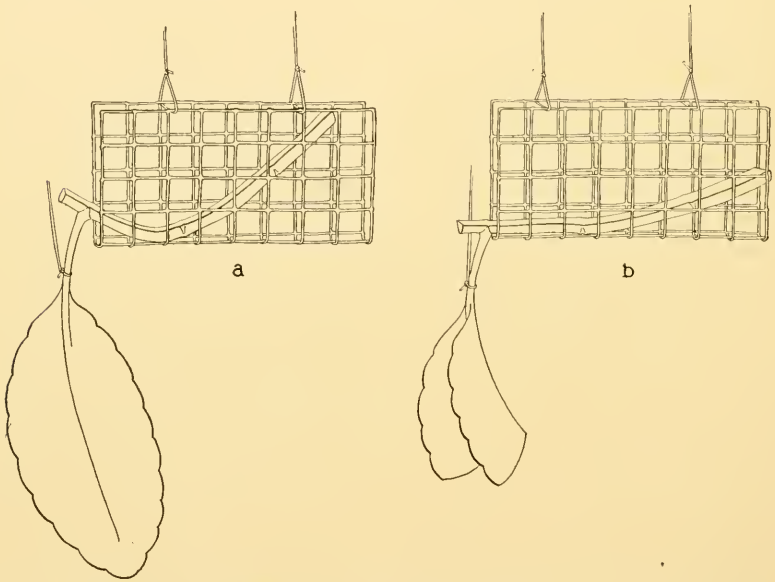

FIG. 55.—Stem split lengthwise, one piece having a whole, the other only half of a leaf at apex. Curvature is greater in stem with whole leaf. May 8 to 11.

three stems increased with the mass of the apical leaf, being a minimum in *c* and a maximum in *a*. When the stem contains no leaf the curvature is a very slow process.

It seemed more accurate to modify the experiment by always comparing the rate of curvature of two halves of a stem split longitudinally as in Fig. 55, *a* and *b*. A stem with a pair of apical leaves was split as accurately as possible in the middle between the two leaves and each half was suspended as shown in the figure. The leaf was on the lower side of the stem as in the preceding experiment, but while one leaf remained intact, part of the other was cut off, so that its mass was only about one-half that

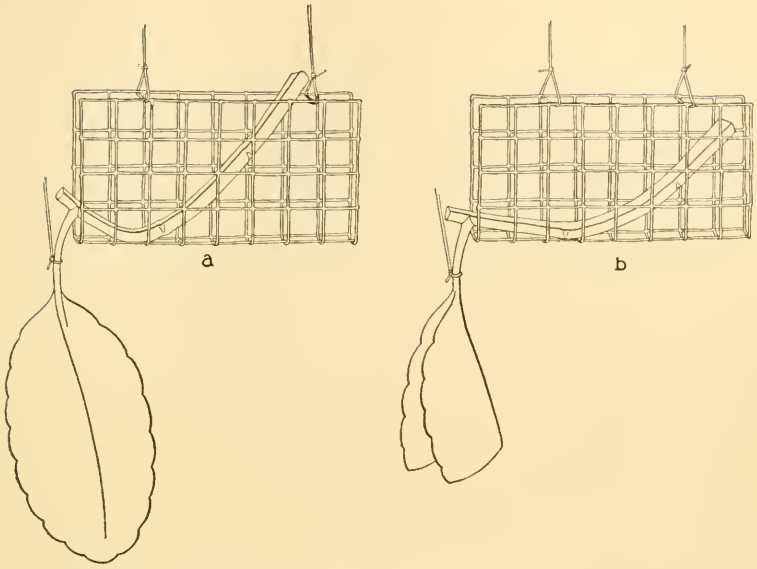

FIG. 56.—The same specimen as in Fig. 55 one day later. Rate of curvature increases with mass of leaf

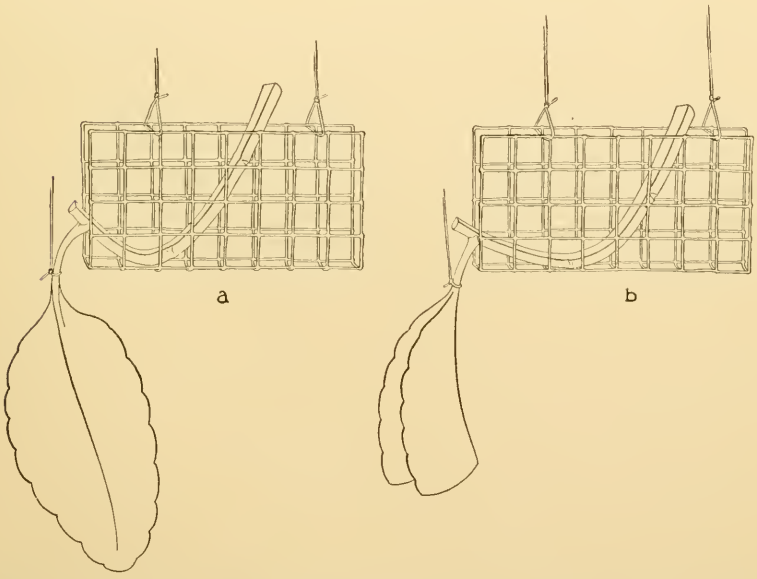

FIG. 57.—The same specimens as in Figs. 55 and 56. Curvature in *b* now almost equal to that of *a*. May 14.

of the whole leaf. Figure 55 gives the curvature after 3 days, the curvature being greater where the mass of the leaf was greater. Figure 56 gives the curvature of the two half stems a day later, and Fig. 57 on the sixth day.

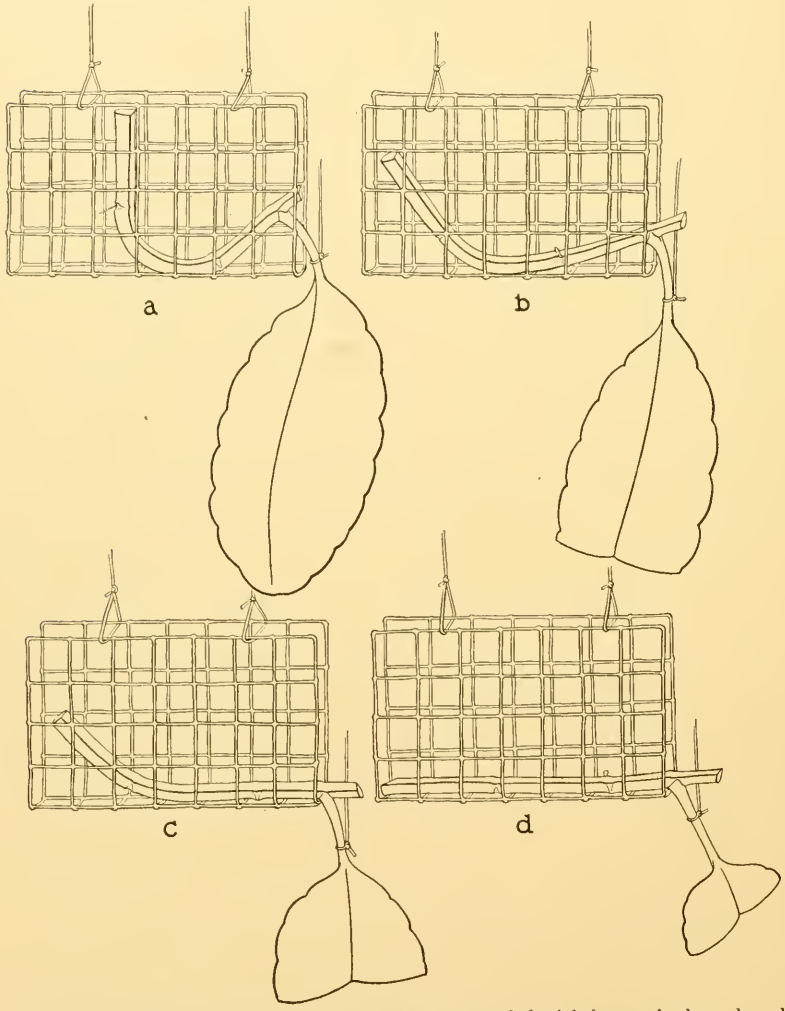

FIG. 58.—*a*, piece of stem with whole leaf; *b*, *c*, and *d* with increasingly reduced leaf. The geotropic curvature increases with mass of leaf. May 8 to 17.

This experiment gives also an idea of the rapidity with which the curvature occurs at the temperature of the greenhouse (about 24°C.).

It may finally be of interest to give some further data showing that the rate of geotropic curvature of these half stems with an apical leaf attached occurs approximately in proportion with the mass of the leaf. In Fig. 58 the curvature of 3 half stems each with a whole leaf was compared with the curvature of the 3 sister half stems each with a reduced leaf. All of the 3 stems with whole leaves had reached about the same curvature as stem *a*, and therefore this one alone is reproduced here. It was found that *b*, *c*, and *d* had bent approximately in proportion with the mass of the apical leaf attached.

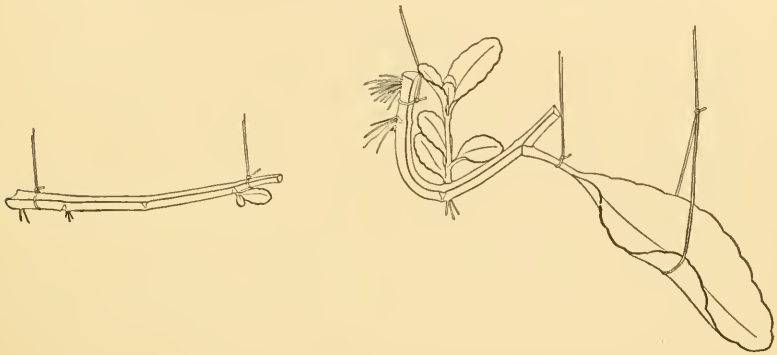

FIG. 59.—Stem split longitudinally and suspended horizontally, one-half is entirely defoliated, the other has a leaf attached to the apex. The latter bent rapidly in the usual way, while the half stem without leaf bent only very slowly. Apr. 10 to May 23.

In Fig. 59 a stem was split longitudinally and both halves were suspended horizontally. One half had a leaf at the apex, while the other half had no leaf. The latter bent very slowly in comparison with the half which had an apical leaf attached, in accordance with the difference in the mass of material available for growth in the two half stems. It may be stated incidentally that the mass of shoots and roots regenerated by the half stem with a leaf was also considerably larger than the mass of shoots and roots formed in the half stem without leaf. The experiment lasted from Apr. 10 to May 23.

The experiments with split stems give on the whole less reliable results than those with whole stems. Not only the unavoidable errors in halving the stem, but possibly other variables vitiate the result, *e.g.*, the unequal degree of drying of the upper side of the stem and the resulting inequalities in rigidity of the wood.

In all the experiments thus far discussed the leaf was at the apex of the stem. When the leaf is at the base of a piece of stem the curvature occurs also though the rate of curvature is apparently less for the same mass of leaf than if the leaf is at the apex. When the leaf is in the middle of the stem the curvature occurs chiefly in the basal part of the stem.

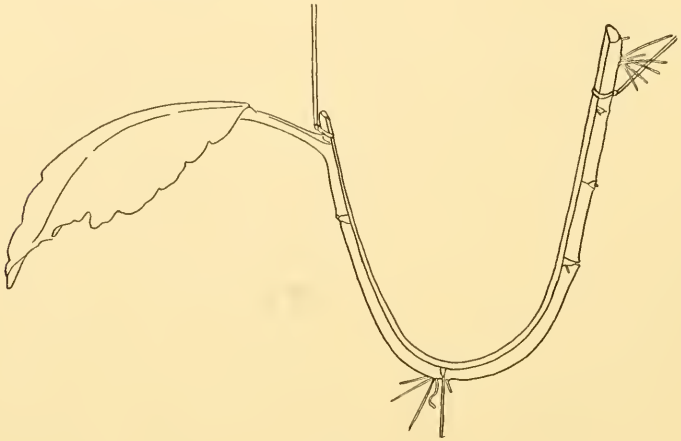

Fig. 60.

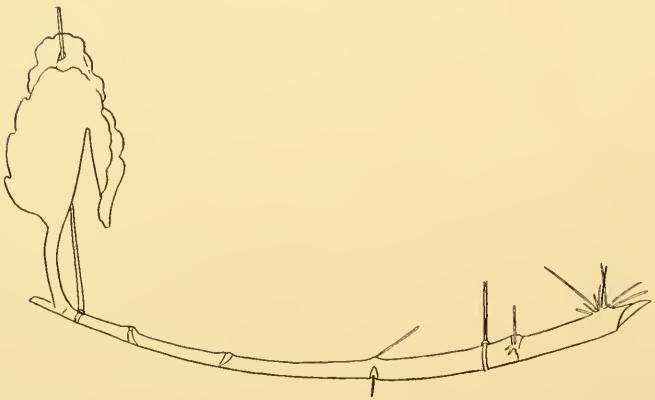

Fig. 61.

Figs. 60 and 61.—Stem split longitudinally, each half having a leaf at the apex. The stems were suspended horizontally in moist air, one with the cortex below (Fig. 60), the other with the cortex above (Fig. 61). Only the half stem with the cortex below (Fig. 60) bends.

3. Mechanism of Geotropic Curvature in *Bryophyllum calycinum*.—In order to find out the mechanism of the geotropic curvature experiments were made with stems split longitudi-

nally. Immediately after the stems were split marks were made with India ink on the cortex at a distance of 1 centimeter from each other and then the stems were suspended horizontally, one-half of the split stems having their cortex below, the others having their cortex above. Stems with an apical leaf were used for the purpose (Figs. 60 and 61). Only when the cortex was below did the stems bend (Fig. 60). After 10 days, when the halves with the cortex below had bent strongly, the displacement of the marks was ascertained. It was found that the marks on the halves in which the cortex was above (Fig. 61) and which had not bent were practically unchanged (Table XVIII). The same was true of the marks in the non-bent regions of the other halves, where the cortex was below; while a growth of 15 to 20 per cent of the original length had taken place in the

TABLE XVIII.—LENGTH OF FOUR SPLIT STEMS PLACED HORIZONTALLY WITH CORTEX ABOVE (IN CENTIMETERS)

At beginning of experiment (June 20)	At end of experiment (July 1)
9.0	9.0
11.0	10.8
10.0	10.0
14.0	13.8

bent convex region of those stems having their cortex below. Table XIX gives the measurements of four bent stems.

TABLE XIX.—LENGTH OF SPLIT STEMS PLACED HORIZONTALLY WITH CORTEX BELOW

Region of stem measured	Set I		Set II		Set III		Set IV	
	Beginning of experiment, centimeters	End of experiment, centimeters	Beginning of experiment, centimeters	End of experiment, centimeters	Beginning of experiment, centimeters	End of experiment, centimeters	Beginning of experiment, centimeters	End of experiment, centimeters
A: non-bent apical part	3.0	3.2	3.0	3.0	4.0	4.0	4.0	4.10
B: bent central part.	4.0	4.9	5.0	5.7	6.0	7.0	4.0	4.85
C: non-bent basal part	2.0	2.0	3.0	3.0	5.0	5.0	4.0	4.15

Figure 62 is a photograph of marked *whole* stems 9 days after the beginning of the experiment. The stems had been suspended horizontally in the aquarium; all had one apical leaf. That part of the cortex which was below had stretched, while the cortex above was shortened. The India ink marks were made at the beginning of the experiment with a distance of 1 centimeter from each other. The photograph shows the change in the position of the marks on the convex side in the bent region of the stem.

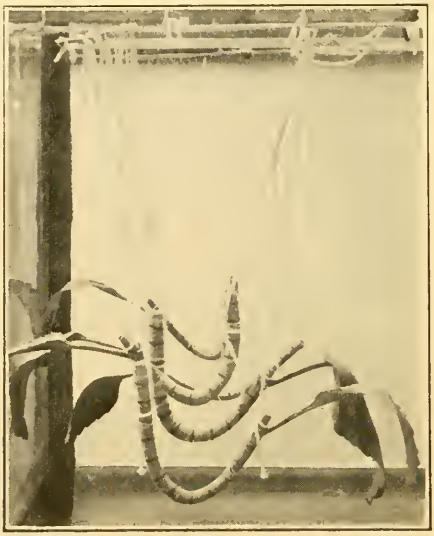

Fig. 62.—Stems suspended horizontally but bent. Marks in India ink show that in the bent region the lower side of the cortex grew in length while the upper side did not. June 3 to 12.

It is highly probable, if not certain, that the increase in length on the lower side of the horizontally placed stem takes place primarily in the cortex of the bending region, not in the wood. This follows from the behavior of these two parts when the cortex of a bent (split or whole) stem is removed, and the rigidity of the cortex is compared with that of the pith and wood taken out.

If we remove the cortex on the lower (convex) side of a split geotropically bent stem, like that in Fig. 60, we find that the rigidity of the cortex in the bent region is much greater than that of the wood; the latter appears soft in comparison with the cortex of the bent region on the convex side of a geotropically bent stem. It is possible also that the increase in the rigidity of the cortex

in this region may be due to a thickening of the cortex, a point which needs further investigation.

4. Influence of Light.—In stems with a large apical leaf the geotropic curvature takes place also in the dark although less rapidly than in the light. In this case the material formed previously and stored in the leaf may be utilized, since the geotropic curvature takes place very rapidly. On account of the fact that light plays less of a role in the case of geotropic curvature of the stem than in the case of regeneration of shoots or roots the suspicion arose that the curvature was only due to a collection of water on the lower side of the stem. To test this idea experi-

FIG. 63.—Geotropic curvature occurs also in stems put under water, though the degree of geotropic curvature seems to be diminished.

ments were made with stems partly under water. It was found that when the bending part of the stem was entirely under water (Fig. 63), the curvature nevertheless occurred though it may have been diminished. This suggests that the curvature was due to the solutes but not exclusively to the water of the sap sent out by the apical leaf into the stem.

The theory of geotropic curvature of the stem of *Bryophyllum calycinum* when put in a horizontal position is then as follows: This curvature is determined by the excess of longitudinal growth of the cortical layers on the under side of the stem and this excess of growth increases with the mass of an apical leaf attached to the stem. The geotropic curvature seems therefore to be a

phenomenon dependent upon the quantity of the material sent by the leaf into the stem or formed in the stem itself.

The fact that the material sent by an apical leaf into the stem collects in greater quantity on the lower than on the upper side of a stem suspended horizontally finds its explanation in the assumption that under the influence of gravity the tissue sap collects on the lower side of such a stem.

It is hardly necessary to state that this explanation is intended merely to account for the observations on the geotropic curvature of the stem of *Bryophyllum*.

CHAPTER XI

THE INFLUENCE OF GRAVITY ON THE POLAR CHARACTER OF REGENERATION IN A STEM OF *BRYOPHYLLUM* (*CONTINUED*)

When a completely defoliated stem is suspended horizontally in moist air the polar character of regeneration is not changed as far as shoot formation is concerned. Shoots continue to form in the apex. The polar character of root formation undergoes, however, a change which becomes striking when the leaves are not all removed. It is true that roots continue to be formed at the extreme base of the stem and this root formation undergoes no change when the stem is put into a horizontal position. The change manifests itself in the fact, however, that in addition to this polar form of root formation there appears now a second form, namely, along the whole lower side of the stem. This latter effect is due to the collection of tissue sap on the lower side of the stem under the influence of gravity. This peculiar influence of gravity on the regeneration of horizontally placed stems finds its explanation in the assumption already referred to that there are two channels for the distribution of sap in the stem; first, by the vascular system, and second, by the gaps between cells and tissues. The sap flow in the vascular bundles is little, if at all, influenced by gravity, and those forms of regeneration which depend upon the vascular sap are little if at all affected by gravity. We notice therefore that shoots continue to form in abundance at the apex and roots at the extreme base of a stem placed horizontally.

The tissue sap, however, follows gravity, collecting on the lower side of the stem where roots but no shoots are formed. In this respect a stem suspended horizontally differs from a leaf which when suspended horizontally forms both roots and shoots on the lower edges. This difference in the influence of gravity on regeneration in the leaf and the stem is a strong argument in favor of the tissue theory of polarity and against the hormone theory. For if it is true that the descending sap which is responsible for the root formation at the base of a stem has this effect because it

reaches primarily root-forming tissue, we should expect that the tissue sap collecting on the lower side of a horizontally placed stem should give rise only to roots but not to shoots, as it actually does.

Figure 64 shows the regeneration which occurs in stems suspended horizontally in moist air. The upper stem is entirely

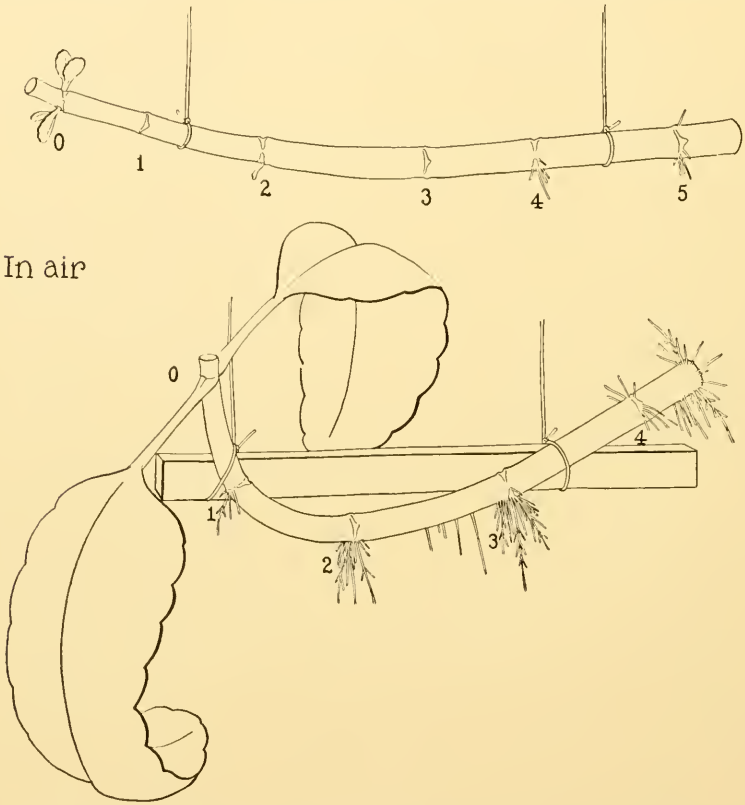

FIG. 64.—Roots grow on under side of stems suspended horizontally, but more roots grow when there is a leaf at the apex of the stem. Nov. 18 to Dec. 13, 1923.

defoliated. It has formed shoots at the apex and roots on the upper as well as on the lower side of the most basal, fifth node, but more on the lower side. There are also a few roots on the lower side in the fourth node and none on the upper. There is only a slight indication of the influence of gravity on the growth of roots. The geotropic curvature is only slight as it always is in completely defoliated stems.

The lower drawing in Fig. 64 gives the influence of a pair of apical leaves on the formation of roots in a stem which was suspended horizontally. Both the upper and lower stems were in the same aquarium and both experiments were carried out simul-

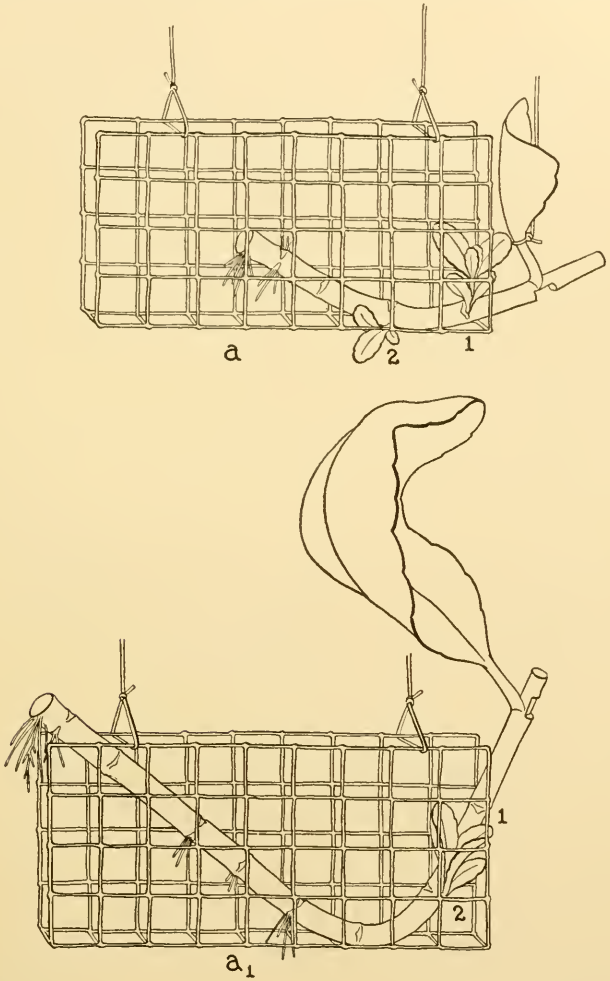

FIG. 65.—The root formation on the lower side is greater when the mass of apical leaf is greater. May 4 to June 5, 1923.

taneously (Nov. 18 to Dec. 13, 1923). At and near the base the lower stem (with two leaves at the apex) forms roots on the upper side as well as on the lower. In nodes 1, 2, and 3, however, and

in the internode between 2 and 3, abundant roots are formed exclusively on the lower side. In order to prevent the stem from undergoing too much curvature and from being thrown altogether out of an approximately horizontal position, it was loosely tied to a piece of wood as shown in the drawing.

It can be shown that the mass of the roots formed in horizontally placed stems increases with the mass of the apical leaf. The two stems in Fig. 65 were suspended simultaneously, the lower stem with a whole leaf at the apex formed more roots than the upper stem with a leaf reduced in size (May 4 to June 5, 1923).

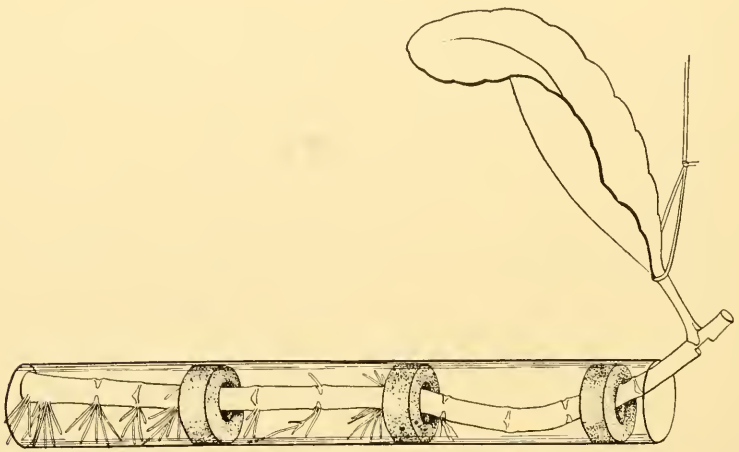

FIG. 66.—Roots grow on under side of a stem suspended horizontally even when the geotropic curvature of the stem is prevented.

It can be shown quantitatively that the formation of air roots in stems suspended horizontally increases with the mass of the apical leaf. These experiments are not very accurate on account of the small weight of the roots formed and on account of the fact that air roots dry out and fall off, while new air roots are formed. Table XX shows, however, that on the whole the mass of roots formed increases with the mass of the apical leaves.

In these experiments the stems bent geotropically and the curvature increased with the mass of the apical leaf. This might suggest the idea that the root formation on the under side of the stem was not a direct effect of the collection of sap on the lower side of the stem but an indirect effect and that it was primarily due to the curvature. This, however, is not correct.

TABLE XX.—SHOWING THAT THE MASS OF AIR ROOTS IN A STEM DIMINISHES WITH THE MASS OF THE APICAL LEAF. THE STEMS WERE SPLIT LONGITUDINALLY, EACH HALF POSSESSING ONE SISTER LEAF AT THE APEX. IN ONE SET THE LEAF WAS LEFT INTACT, IN THE OTHER IT WAS REDUCED IN SIZE BY CUTTING OFF PART OF THE LEAF. THE STEMS WITH REDUCED LEAF INVARIABLY FORMED A SMALL MASS OF ROOTS

No. of experiment	Date and duration of experiment		Weight of leaves, grams	Weight of air roots on stem, grams	Approximate ratio of weight	
					Of leaves	Of roots
1	Jan. 15 to Feb. 21, 1918.	6 whole leaves, *fresh*	19.03	0.054	6.7	6.7
		6 sister leaves reduced in size, *fresh*	2.85	0.008		
2	Oct. 29 to Dec. 9, 1918.	5 whole leaves, *fresh*	51.1	0.077		
		5 whole leaves, *dry*	2.627	0.016	3	3
		5 sister leaves reduced in size, *fresh*	16.33	0.025		
		5 sister leaves reduced in size, *dry*	0.901	0.005		
3	Jan. 9 to Feb. 17, 1919.	5 whole leaves, *fresh*	11.65	0.043		
		5 whole leaves, *dry*	0.526	0.006	4	2
		5 sister leaves reduced in size, *fresh*	2.935	0.022		
		5 sister leaves reduced in size, *dry*	0.132	0.003		
4	Jan. 22 to Mar. 6, 1919.	6 whole leaves, *fresh*	34.462	0.128		
		6 whole leaves, *dry*	1.378	0.037	3.3	6.1
		6 sister leaves reduced in size, *fresh*	10.032	0.026		
		6 sister leaves reduced in size, *dry*	0.416	0.006		
5	Jan. 31 to Mar. 3, 1919.	5 whole leaves, *fresh*	32.8	0.044		
		5 whole leaves, *dry*	1.504	0.009	3	2
		5 sister leaves reduced in size, *fresh*	8.625	0.026		
		5 sister leaves reduced in size, *dry*	0.485	0.005		
6	Oct. 27 to Dec. 9, 1918.	5 whole leaves, *fresh*	14.4	0.423		
		5 whole leaves, *dry*	1.36	0.109	11.6	6.4
		5 sister leaves reduced in size, *fresh*	1.57	0.900		
		5 sister leaves reduced in size, *dry*	0.117	0.017		

When the stems are prevented from geotropic bending as in Fig. 66 roots are nevertheless formed in abundance on the lower side of the stem. And, furthermore, if the stem is bent passively (by tying it to a piece of wood) so that its convex side is directed upwards as in Fig. 67 the roots form nevertheless on the lower side. This all is in harmony with the assumption that the excess of root formation on the lower side of a stem suspended horizontally in moist air is due to a collection of tissue sap on the lower side of the cortex of the stem.

Now if the sap sent out by the basal leaf in the ascending direction is as capable of causing root formation as is the descending sap, we should be able to demonstrate that when a stem possessing a pair of leaves at the base is suspended horizontally, roots will also form on the lower side of the stem. That this is the case is shown in Fig. 68. The stem had a pair of large leaves at the base, one of which dipped into water. In order to prevent excessive geotropic curvature the stem was loosely fastened to a

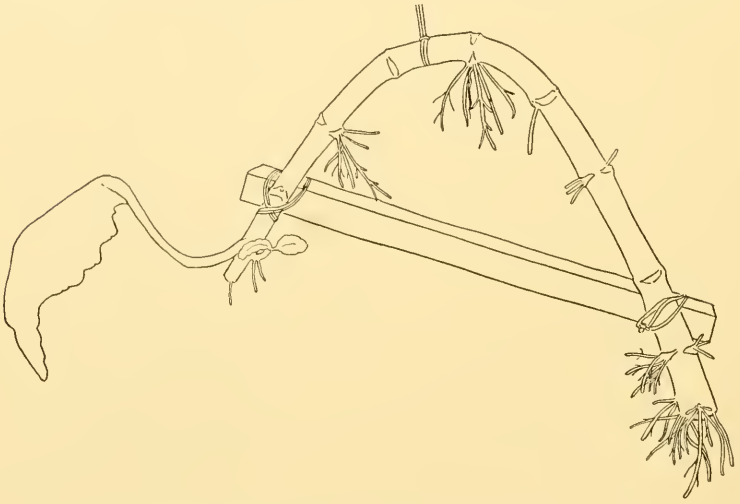

Fig. 67.—Stem bent passively with convexity on upper side. Roots grow on the lower concave side of the stem.

piece of wood. Nevertheless some geotropic curvature occurred. The stem has formed no roots on the upper side but an abundant mass of roots on the lower side and some on the sides. The drawing was made on the eleventh day of the experiment. Since the same stem without a leaf would in this time have formed practically no roots on the lower side, the enormous root formation on the lower side of the apical stem must be due to the ascending sap sent out by the basal leaves. The same results were obtained in a large number of experiments though the root formation varies in different stems, especially with the mass of the leaf. But the same is true for the root formation caused by the apical leaf.

It follows from this that the ascending sap sent out by a leaf gives rise to roots if it reaches cells capable of forming roots;

and that the polar character of regeneration in the stem of *Bryophyllum* must therefore be due to the fact that in a stem suspended upright and vertically the ascending sap flows in channels which reach primarily the anlagen for shoot formation but not those for root formation.

FIG. 68.—Stems with leaves at the base and suspended horizontally form roots apically from the leaf on the under side of the stem, but not on the upper side, showing that the ascending sap from a leaf can form roots as well as shoots. Dec. 16 to 27, 1923.

When the stem is suspended vertically the tissue sap causes the initial formation of roots in nodes above the basis which was described in Chapter VIII.

When a leaf is in the middle of a stem suspended horizontally the main curvature occurs chiefly in the basal parts of the stem as does also the root formation.

This influence of gravity on the formation of roots in a stem of *Bryophyllum* suspended horizontally is more pronounced when

the stem is suspended in moist air than when suspended under water. In this case roots appear in each node, but sometimes without respect to gravity (Fig. 63). A similar disappearance of the effect of gravity on the formation of organs was noticed in Chapter V when leaves were put under water. The explanation given there may hold also for stems, namely that the effect of gravity is primarily due to tissue sap collecting on the under side of a stem suspended horizontally. This causes an acceleration of growth on the under side which in its turn causes a flow of sap of the whole stem towards the under side, thereby suppressing the growth of roots on the upper side of the stem. When, however, the stem is under water the collection of the water of the tissue sap on the lower side of the stem can no longer cause a more rapid growth of roots on this side, since the tissues on the upper side of the stem have also an abundance of water.

CHAPTER XII

THE INHIBITORY ACTION OF APICAL LEAVES ON THE FORMATION OF SHOOTS IN THE LOWER PART OF THE STEM

1. There exists a group of phenomena which allows us to put the hypothesis of polarity in regeneration developed in the preceding chapters to a further test. These phenomena are concerned with the inhibition of shoot formation in a stem by leaves at the apex of the stem. This inhibition is observed in the stems of young plants of *Bryophyllum* (less than one year old) when suspended vertically and upright, either in moist air or with their bases in water. When the stems of old plants are used or when the stems are suspended horizontally, different results are obtained which will be discussed in their proper place.

In order to understand these phenomena attention should once more be called to the arrangement for the anlagen of shoots in stems of *Bryophyllum*. In the axil of each leaf of a stem there exists one bud capable of giving rise to a shoot. Each node of a plant has 2 leaves in opposite position, and the axis connecting the 2 axillary buds in one node is always at right angles with the axis connecting the two buds of the next node (Fig. 69). Thus the line connecting the two buds at node 2 (Fig. 69) is at right angles with the line connecting the two buds in node 1 as well as in node 3, etc. The lower leaves on a stem fall off in time, leaving their axillary buds exposed.

In Fig. 70 the path of the sap from an apical leaf is indicated by shading. The sap from one leaf goes through the anlagen for both shoots in the first and third node below the leaf, and through the anlagen of one shoot in the second and fourth node below the leaf; namely, on the side of the apical leaf. The buds in the second and fourth node below the apical leaf and on the opposite side of the stem alone are not in the path of the descending sap from the leaf and we shall find that they alone can grow into shoots.

We have seen that when a piece of defoliated stem is suspended in moist air with its base in water shoot formation commences in a few days in the most apical nodes and the apical shoots grow rapidly. When, however, the piece of stem of a plant less than one year old is defoliated, leaving only a pair of leaves at the apex (Fig. 71), and if the stem is suspended vertically, roots are formed in abundance at the base but all the shoot formation in this stem is suppressed for a long time if not indefinitely. The draw-

Fig. 69.

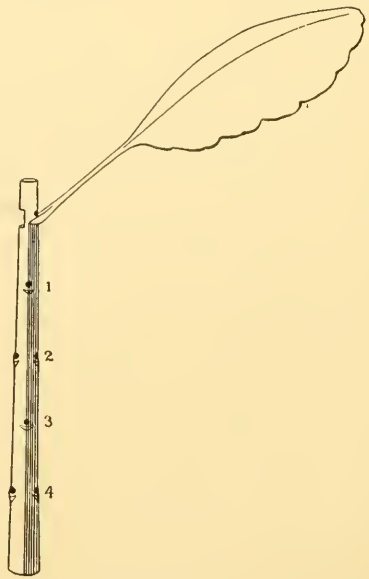

Fig. 70.

Fig. 69.—Diagram showing arrangement of shoot buds in the stem of *Bryophyllum calycinum*. The line connecting the two buds in one node is always at right angles to the line connecting two buds in the next node.

Fig. 70.—Diagram explaining the inhibitory influence of the descending sap from the apical leaf on shoot formation in the stem. The region of the stem reached by the sap from the apical leaf is shaded, and in the shaded part regeneration of shoots is inhibited.

ing was made after 6 weeks, but the situation had not changed in the next month when the experiment was discontinued. One stem in six finally formed a shoot in the axil of one of the apical leaves and one in the fourth node below the apical leaves. This experiment shows that the descending sap from a leaf favors root formation but suppresses shoot formation in the basal part of a young stem. This fact is also shown in the following experi-

ments: If all the leaves of a piece of stem less than one year old, with the exception of one sufficiently large apical leaf, are removed (Fig. 72) and if the bud opposite the leaf in the most apical node (designated as 0) is also removed, no shoots are formed in node 1 (that is, the first node below the leaf), but one

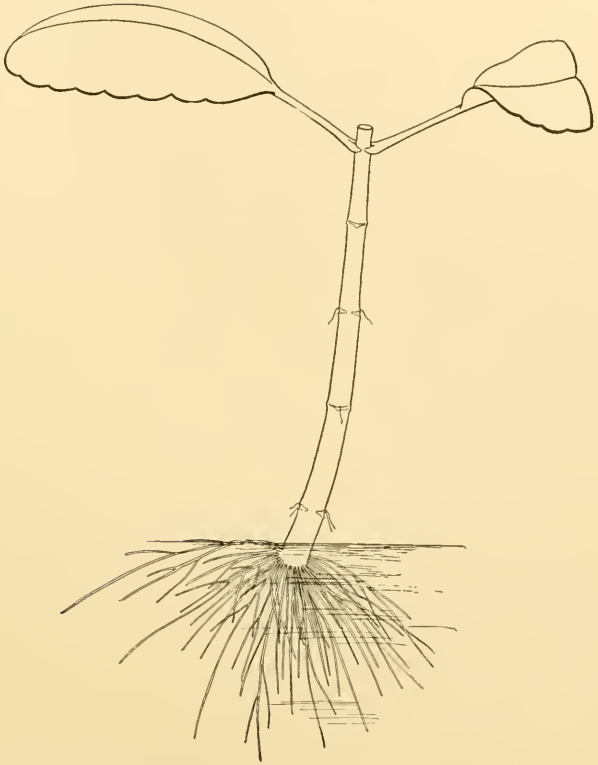

FIG. 71.—Inhibitory action of apical leaves on shoot formation in the basal part of young stems. Only roots but no shoots are formed in such cases. Oct. 8 to Nov. 19, 1923.

of the buds in node 2 grows; namely, the one on that side of the stem which is opposite the leaf. If this second bud is also removed either a shoot grows out in the fourth node below the leaf, but on the side of the stem opposite to that of the leaf, or (what is more generally the case) no regeneration occurs in the stem.

These observations confirm the fact that the descending sap from the leaf inhibits all shoot formation in its path, that is,

on that side of the stem where the leaf is, while on the opposite side which is not reached by the descending sap from the leaf a shoot can form. The two buds in the first and third node below the leaf, being in the path of the descending sap from the leaf, are prevented from growing while the bud in the second or fourth node below (Fig. 72) but opposite the leaf is outside the path of the descending sap from the leaf and can therefore develop.

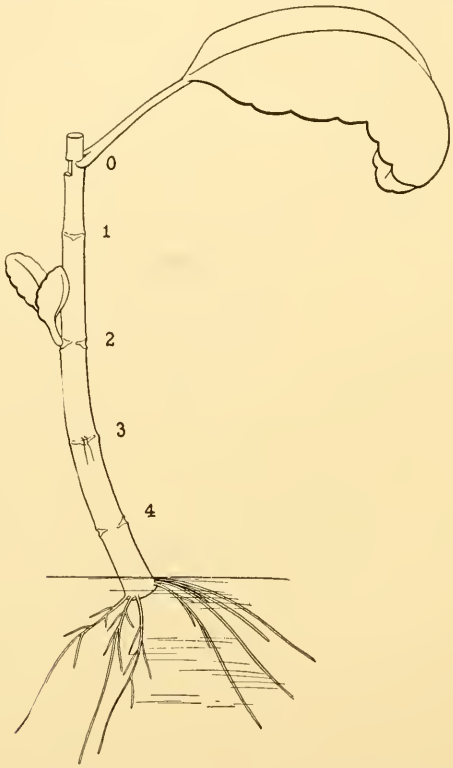

FIG. 72.—Young stem with one apical leaf. Bud opposite the leaf (in node 0) is cut out. Shoot formation in the first node below the leaf is suppressed, but a shoot is formed in the second node below and opposite the leaf.

That the descending sap from a leaf inhibits shoot formation in young stems is further supported by the fact that when the size of the apical leaf is sufficiently reduced the inhibitory effect of the leaf on shoot formation ceases, and that shoots can now grow out in the first node below the leaf (Fig. 73). And finally, if half of the base of the petiole of the apical leaf (where it joins

the stem) is removed, and if also the bud opposite the leaf is removed (Fig. 74), one of the two buds for shoot formation in the first node below the leaf can grow out, namely the one on the side where the half of the base of the petiole is removed. In this case the path of the descending sap from the leaf goes through only one of the two buds in the first node below the leaf and in this alone the shoot formation is inhibited.

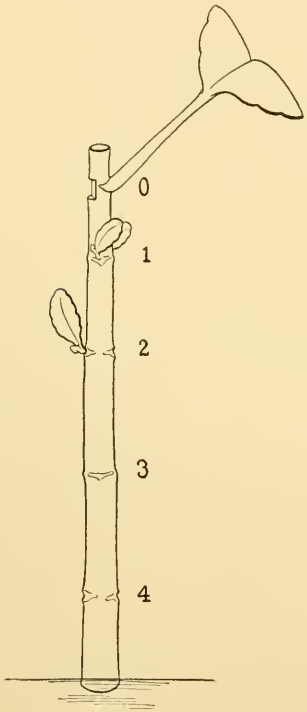

FIG. 73.

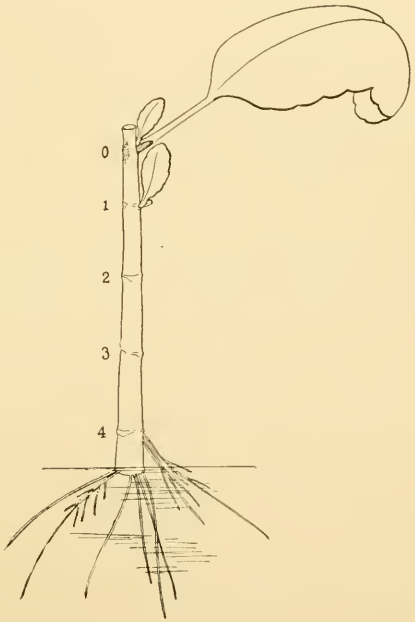

FIG. 74.

FIG. 73.—Young stem with only a small piece of one leaf at apex. In this case the shoot formation in the node below the leaf is no longer suppressed. (Bud opposite leaf in node 0 is cut out.)

FIG. 74.—Half of the base of the petiole of leaf cut out. In this case one of the two buds in the first node below the leaf can grow out; namely, on that side where the base of the petiole of the leaf is removed. (Bud opposite leaf in node 0 is cut out.)

The question arose whether basal leaves can overcome the inhibiting effect of apical leaves on the regeneration of the stem. This is possible when there is only one leaf at the apex but not when there are two. Thus a stem with a pair of leaves in the

apical node and in the second node below the apex will form no shoots in the first node below the apex (Fig. 75). The inhibitory effect of the two apical leaves is too great. When, however, there is only one leaf in the apical node and one leaf in the second node below, but on the opposite side of the stem, and if in addition the bud opposite the apical leaf is cut out, the shoots in the first node below the apical leaf may grow out (Fig. 76). The inhibitory effect of one apical leaf on the buds in the first node

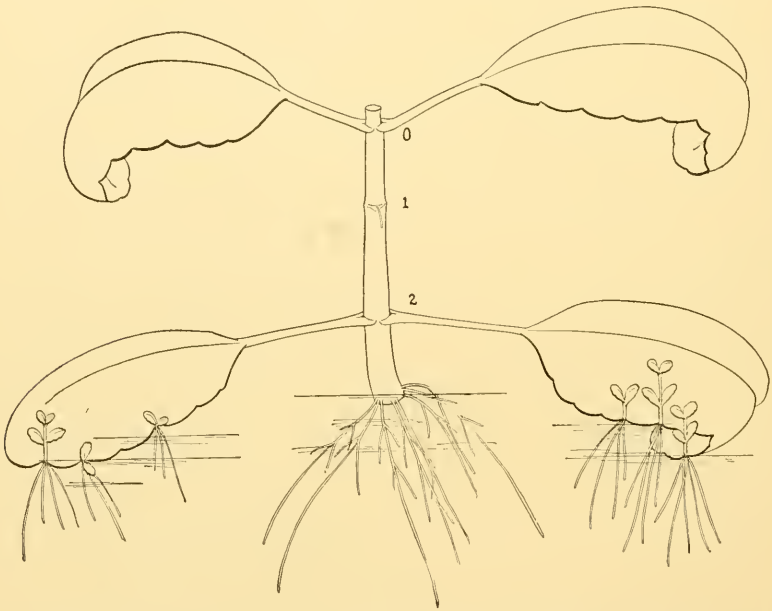

FIG. 75.—One pair of leaves at apex and one pair at second node below, in a young stem. The influence of the basal leaves does not suffice to overcome the inhibitory effect of the two apical leaves on the shoot formation in the node in the middle of the stem. Nov. 20 to Dec. 17.

below the apical leaf is only half of that of two leaves (of the same size) and this inhibition can be overcome by a basal leaf.

When this experiment is repeated with stems in which the buds in the first node below the leaf are cut out, the axillary shoot of the leaf at the apex will grow out though usually with some delay (Fig. 77).

In all these experiments the inhibitory effect of the apical leaf is stronger for those anlagen of shoots which lie in the middle of the path of the descending sap from the apical leaf than for those

FIG. 76.—One leaf at apex of young stem and one in second node below on opposite side of stem. The inhibitory effect of only one apical leaf is overcome by the basal leaf. Oct. 10 to Nov. 2.

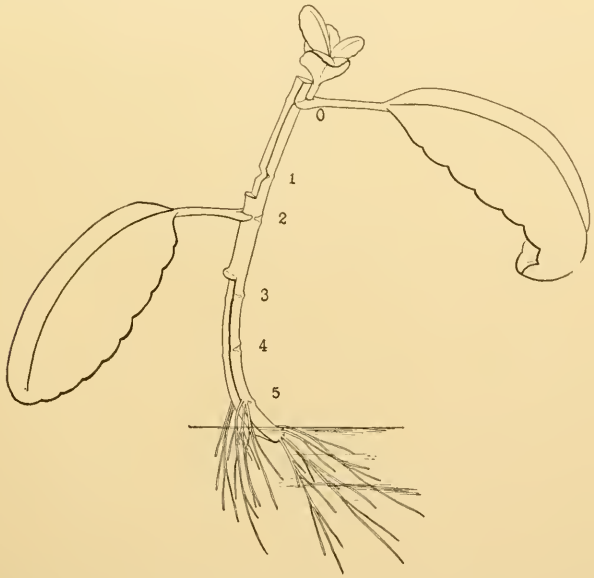

FIG. 77.—Same experiment as in Fig. 76 except that the buds in node between the two leaves are cut out. The shoot in the axil of the apical leaf now grows out. Oct. 15 to Nov. 23.

anlagen which lie a little more on the side. This is clearly illustrated in experiments on old stems split longitudinally and possessing one apical leaf (Fig. 78). In this case the shoots in the first and third node below the leaf can grow out but not the shoot in the second node below the leaf, because the latter is in the middle of the path of the descending sap from the leaf while the other buds are only on the side.

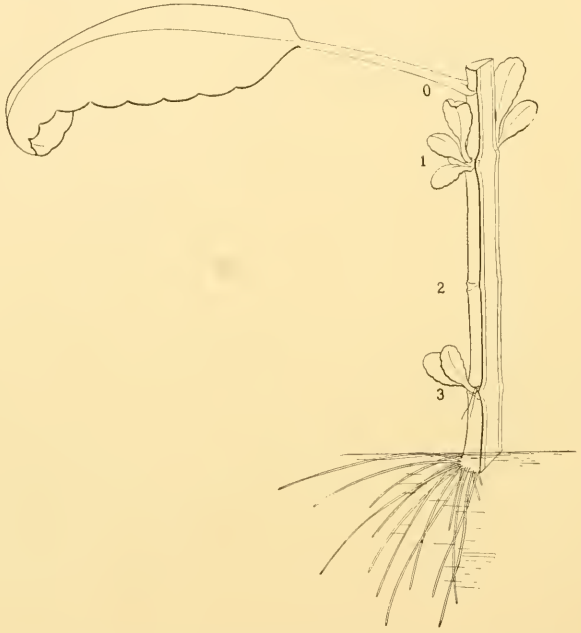

Fig. 78.—Old stem split longitudinally, one leaf at apex. In this case shoot formation occurs in first and third node below the apical leaf, but not in the second node below the leaf. Oct. 24 to Nov. 13.

2. These experiments leave no doubt that the sap sent out by an apical leaf in the descending current of sap inhibits the shoot formation in the parts of the stem below the leaf which lie in the path of the descending sap; provided the stems are suspended vertically and are young (less than one year old). There are, as far as the writer is aware, two ways of accounting for this inhibitory action of the descending sap from the leaf on shoot formation below. The first is that the descending sap contains some specific hormone which directly suppresses shoot formation and favors root formation. This idea is, however, in contra-

diction with the theory of polarity at which we arrived in the preceding chapter.

The second is that the inhibitory action of the descending sap from the leaf on shoot formation in the basal part of the stem is an indirect one and due to the fact that this sap is either consumed entirely for the growth of the stem in length or in thickness

FIG. 79.—In old stems the apical leaves no longer inhibit shoot formation below.

and that for this reason none or too little of the material contained in the descending sap is available for shoot formation; or that the rapid growth of certain parts of the stem attracts the sap from the anlagen for shoots in this region and thereby inhibits shoot formation; or that this rapid growth of the stem inhibits the shoot formation indirectly in some other way. Such an explanation would be in harmony with the theory of polarity of regeneration given in the preceding chapter. These phenomena of

inhibition of regeneration offer therefore a good test of our views concerning the cause of polarity in regeneration.

As long as we are satisfied with purely qualitative experiments the hypothesis of inhibitory hormones sent by the apical leaf in the descending sap seems to be adequate, although we already meet with a difficulty in the fact that these phenomena of inhibition are found only in young stems still growing vigorously, but not in old stems. Thus in the old stem in Fig. 79 the two apical

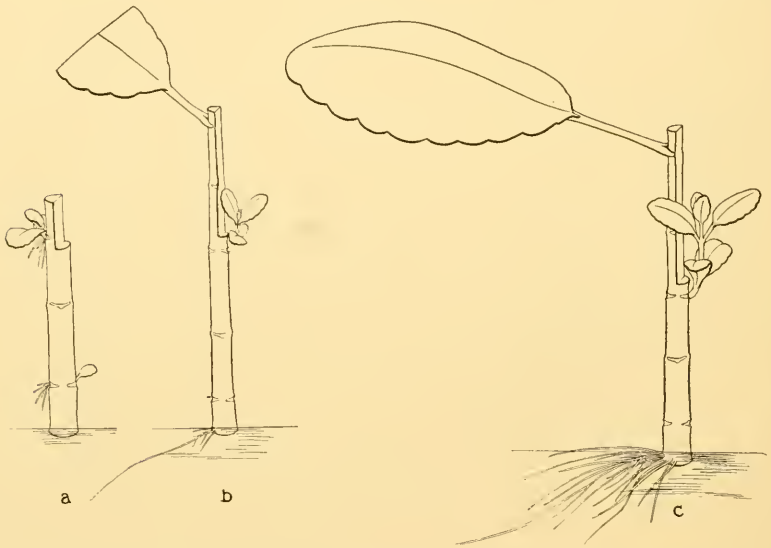

FIG. 80.—Proof that the descending sap from a leaf can also under proper conditions increase shoot production. The shoot production in *c*, with a whole apical leaf attached, is greater than in *b*, with only a piece of leaf attached. Without leaf, in *a*, the shoot production is a minimum. Roots at the base form in *b* and *c* first on that side of the stem where the leaf is. Dec. 7 to Jan. 5, 1923.

leaves did not inhibit the growth of vigorous shoots in the first node below the apical leaves. The fact that the inhibitory effects of the apical leaf described in younger stems are less obvious or non-existent in older stems (Figs. 78 and 79) would already demand a modification of the hypothesis. The difficulties increase when we supplement the qualitative by quantitative experiments.

3. It can be shown quantitatively that the descending sap sent out by the apical leaf will under proper conditions increase the shoot formation in the basal part of the stem.

In Fig. 80, *a* is a piece of stem without leaf, *b* a piece of stem with a reduced piece of leaf attached to the apex, and *c* a piece of stem with a whole leaf attached to the apex. The right side of the upper part of the stem opposite the leaf is cut off. The stems of *b* and *c* have formed roots at the base on that side of the stem where the leaf is, showing that the material for root formation was carried in the descending current from the leaf. Moreover, the mass of roots is greater in *c* than in *b*, corresponding to the difference in the mass of the leaf. Later on, however, roots may form in the whole circumference of the base of the stem. Stem *a*, which had no leaf, has not formed any roots at the base, but only the transitory air roots in nodes, these air roots disappearing when the permanent roots at the base are formed. No shoots were formed in *b* and *c* on the side of the stem where the leaf is, showing the inhibitory effect of the descending sap, but shoots were formed on the side opposite to that of the leaf. The mass of shoots is greatest in *c* where the mass of the leaf is greatest, is smaller in *b* where the leaf is reduced in size, and smallest in *a* where there is no apical leaf.

All these experiments were carried on simultaneously and lasted from Dec. 7, 1922 to Jan. 5, 1923.

The determinations of the dry weight show that the mass of shoots produced per gram of dry weight of stem increases with the size of the apical leaf, and that therefore the material of which the shoots in *b* and *c* are formed is partly furnished by the descending sap from the leaf (Table XXI).

TABLE XXI

	Dry weight of stems, grams	Dry weight of shoots, grams	Dry weight of basal roots, grams	Weight of shoots produced per 1 gram of stem, milligrams
a. Six stems without leaves.....	3.252	0.070	0	21.5
b. Four stems with reduced leaves..	1.883	0.059	0.014	31.0
c. Five stems with whole leaves....	3.934	0.180	0.074	46.0

The dry weight of the mass of leaves in *b* was 0.470 gram, in *c*, 2.607 grams.

The increase in the shoots produced from the descending sap from the apical leaf increased with the mass of the apical leaves, but less rapidly. (The mass of the basal roots increased, however, almost in direct proportion with the mass of the leaf.)

Part of the material sent out by the leaf in the descending current seems to be utilized for the growth in length and thickness of the more peripheral tissues of the stem from which the roots originate, leaving only a fraction of the material of the descending current free to be utilized for the growth of the shoots in *b* and *c*

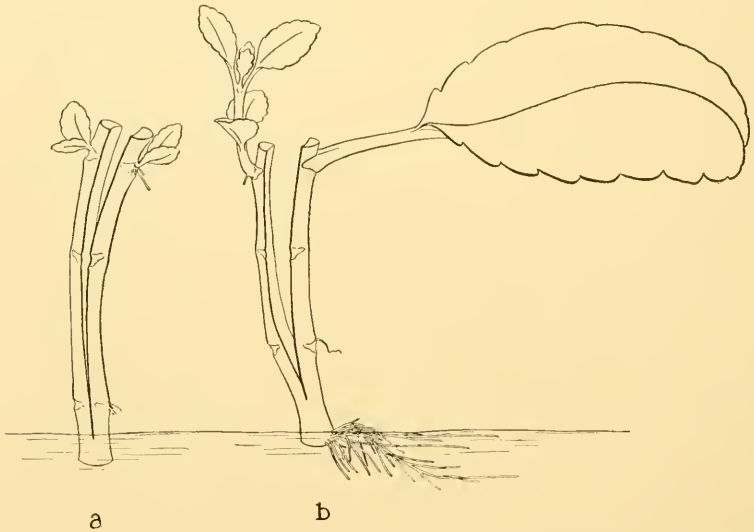

FIG. 81.—Stems split longitudinally from apex almost to the base. Stem *b*, with one apical leaf, forms a shoot with greater mass than the stem *a*, without leaf. In stem *b* the shoot formation is suppressed on the side where the apical leaf is. Root formation in *b* first on side of leaf. Jan. 12 to Feb. 8, 1923.

of Fig. 80. This would explain why the mass of shoots formed in the basal parts of a stem cannot increase in direct proportion with the mass of the apical leaf.

That the descending current from a leaf carries material that can be utilized for shoot formation can also be demonstrated in the following form of experiment:

In Fig. 81 the two stems *a* and *b* are split longitudinally from the apex down to near the base which dips into water. Stem *a* has no leaf while stem *b* has an apical leaf to the right. The

experiment lasted from Jan. 12 to Feb. 8, 1923. Stem *a*, without a leaf, formed two tiny shoots at the apex. Stem *b* formed roots at the base on that side only where the leaf is, and a large shoot at the apical end of the stem on the opposite side of the leaf. This shoot exceeded in mass the two tiny shoots formed in *a*. The determinations of the dry weight confirmed this (Table XXII). The mass of shoots formed by the stems alone was only 44 milligrams, while the stems with an apical leaf formed shoots with a dry weight of 358 milligrams. Hence more than seven-eighths of the mass of shoots in *b* was furnished by the descending sap from the leaf at the apex. This sap inhibited the shoot formation on the side where the leaf was and at the same time increased the mass of shoots regenerated by about 700 per cent; hence it cannot be said that the inhibiting action of the descending sap sent out by the leaf is due to an inhibitory substance in the leaf.

TABLE XXII

	Dry weight of stems, grams	Dry weight of regenerated	
		Shoots, grams	Roots, grams
I. Six stems with apical leaf, *b*....	2.802	0.358	0.121
II. Five stems without leaves, *a*....	2.462	0.044	0.002

The dry weight of the 6 apical leaves of Set I was 3.388 grams.

In the preceding experiments the stems dipped into water. The results remain the same when the stems are entirely in air. Three sets of 4 small stems each were split longitudinally and suspended horizontally in moist air (Fig. 82). Set I had a large leaf at the apex, Set II a reduced leaf, and Set III had no leaf. All produced shoots at the upper apical node, but, as Fig. 82 shows, the size of the shoots increased with the size of the apical leaf. In this case, the sap from the leaf had to travel in the descending current to the base of the stem and then on the other side of the split back to the apex. There can be no doubt that the descending current from the apical leaf favored shoot formation. In Set I, 1 gram dry weight of stem produced 57 milligrams dry weight of shoot, in Set II, 37 milligrams, and in Set III, which had no leaf, only 12 milligrams.

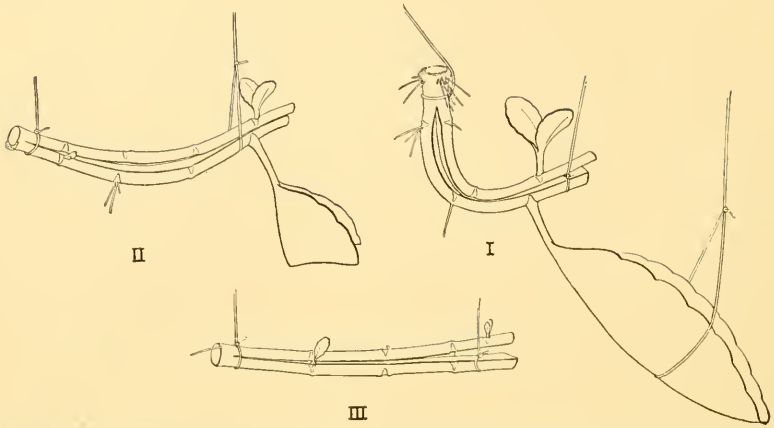

FIG. 82.—Similar experiments as in Fig. 81, except that stems and leaves are suspended horizontally in moist air: I, with whole leaf, forms a larger shoot at apex than II, with a leaf reduced in size; III, without leaf, produces only tiny shoots. Notice also that geotropic curvature of stem increases with mass of leaf. Apr. 3 to 21, 1923.

FIG. 83.—A stem partially split. The sap from the apical leaf had to cross over to the other side of the stem and inhibited the shoot formation on this side. Then the sap had to cross over again at the base, giving rise to a shoot. The mass of these shoots was greater in stems with leaf attached than the mass of shoots in stems without leaves. Jan. 9 to Mar. 16, 1923.

The following objection can be raised to these experiments; namely, that the descending sap sent out by the leaf in crossing over to the other side of the stem underwent a change, consisting in the loss of the inhibitory substance. To test the possibility of such a modification of the sap in crossing over from one side of the stem to the other, the following experiment was made:

Stems with one leaf left at the apex were split lengthwise at some distance beneath the leaf, until near the base, which dipped into water (Fig. 83, *a*). The apical bud opposite the leaf was removed as indicated diagrammatically in the figure. In this case the sap from the leaf had to cross over to the opposite side and then to flow down the stem on the side opposite the leaf and on this side the first roots developed at the base, spreading finally, however, all around the base. No shoot developed on that side (Fig. 83, *a*), showing that in crossing over the sap had not lost its inhibitory power. A shoot developed, however, on the same side where the leaf is, at the apex of the split part of the stem (Fig. 83, *a*). Now the question arose, whether or not the descending sap of the leaf contributed to the growth of this shoot. This turned out to be true as the following quantitative experiment shows:

Five stems without leaves were split lengthwise down to near the base, as shown in Fig. 83, *b*, each split half producing a shoot at the apex. Six stems of almost the same mass but with one leaf left at the apex were split as indicated (Fig. 83, *a*). The latter stems produced one shoot at the apex of the split half of the stem as indicated in Fig. 83, *a*, and occasionally a second smaller shoot in the node below. Now the total dry weight of shoots produced by the six stems in *a* was 1.557 grams, while the total dry weight of shoots produced by the five stems in *b*, without leaves, was only 0.668 gram. The dry weight of the six apical leaves was 2.063 grams, about the same as that of the stems. This removes all doubt that the descending sap from the apical leaf contributed to the growth of the shoots below the leaf in *a*. In this case, the descending sap had to travel the whole length of the stem on the side opposite the leaf (Fig. 83, *a*), and had then to ascend again to the apex of the split part of the stem. The exact figures are given in Table XXIII. The mass of regenerated shoots is so great because the experiment lasted longer than usual; namely, over two months (Jan. 9 to Mar. 16).

TABLE XXIII

	Dry weight of stems, grams	Dry weight of regenerated	
		Shoots, grams	Roots, grams
a. Six stems with apical leaf.....	2.139	1.557	0.306
b. Five stems without leaves.....	1.987	0.668	0.040

Two facts have now been proved; first, that the *ascending* sap sent out by a basal leaf can produce roots in a stem of *Bryophyllum* and the quantity of roots produced is smaller when the stem is completely defoliated than when it has one or two leaves at the base; it can also be stated, though this was not proved quantitatively, that the root formation caused by the basal leaf increases with the mass of the latter. Second, that the *descending* sap sent out by an *apical* leaf increases shoot formation in the basal part of the stem and that this shoot formation increases with the mass of the apical leaf. This makes it impossible to attribute the polar character of regeneration in a stem of *Bryophyllum* to differences in the chemical constitution of the ascending and descending sap. The only alternative is, as far as the writer is aware, that the ascending and descending sap reach primarily different kinds of tissues, the ascending sap reaching primarily the anlagen for shoot formation and the descending sap the anlagen for root formation. The fact that the descending sap from an apical leaf inhibits shoot formation and favors root formation in its downward path must be correlated with this conclusion. It was suggested earlier in this chapter that this inhibition might be the indirect result of a growth of the stem in length or thickness as a consequence of which the growth of shoots is inhibited in the basal part of the stem.

CHAPTER XIII

CALLUS FORMATION

In order to explain the inhibitory effects of the descending sap on shoot formation in its path in the way suggested at the end of Chapter XII, it would be necessary to show first that the descending sap causes a growth in the stem which the ascending sap does not produce. The callus formation can be used for this proof since it occurs only at the basal, never at the apical end of a stem of *Bryophyllum*. It can be shown that there is a parallelism

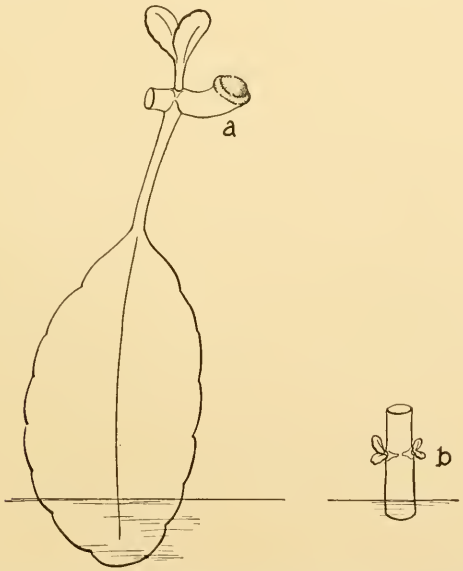

FIG. 84.—Influence of leaf on callus formation in stem. Stem *a*, with leaf, forms callus, while stem *b*, without leaf, does not during the same time. Nov. 15 to Dec. 12.

between the conditions which inhibit shoot formation and those which favor callus formation in a stem.

A small piece of stem without leaf forms little or no callus while at the same time a piece of stem of the same mass with a leaf attached to it forms considerable callus (Fig. 84). In this experiment the piece of stem without leaf had even the advantage of

dipping into water. When kept in air, small pieces of stem without leaf form little or no callus. When a piece of stem with one pair of sister leaves is split longitudinally and one leaf is reduced in size (Fig. 85) the stem connected with the smaller piece of leaf produces in the same time and under the same conditions less callus at the base than the piece of stem connected

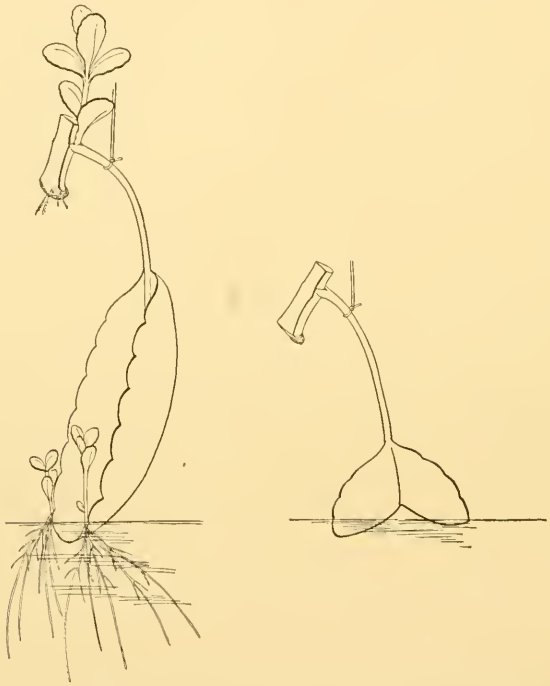

Fig. 85.—Stem connected with whole leaf forms more callus than stem connected with sister leaf reduced in size. Nov. 15 to Dec. 12.

with the larger piece of leaf. The experiment lasted from Nov. 15 to Dec. 12.

The callus formation at the base of a piece of stem increases therefore with the mass of the apical leaf, as does also the inhibiting influence of an apical leaf on shoot formation in its path.

When the apical leaf is near the base the callus formation occurs more rapidly and is larger than when the apical leaf is further from the base; since in the latter case the descending sap from the leaf has a greater distance to travel before it reaches the base and part of its material should be consumed by the stem before it reaches the base. This is demonstrated by Fig. 86

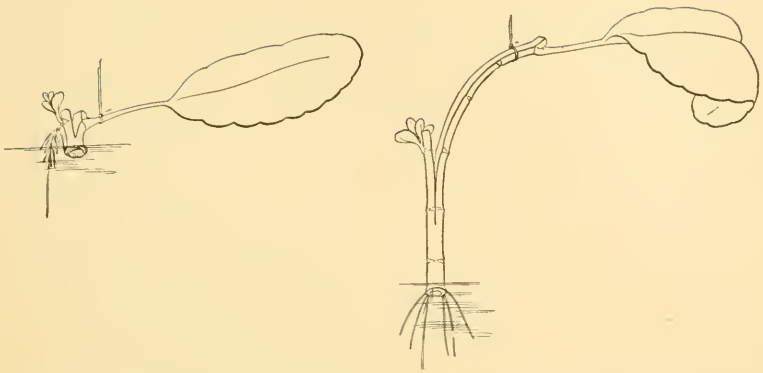

FIG. 86.—When the apical leaf is near the base of the stem (left), callus is formed more rapidly than when apical leaf is at greater distance from the base of stem. Nov. 30 to Dec. 14, 1923.

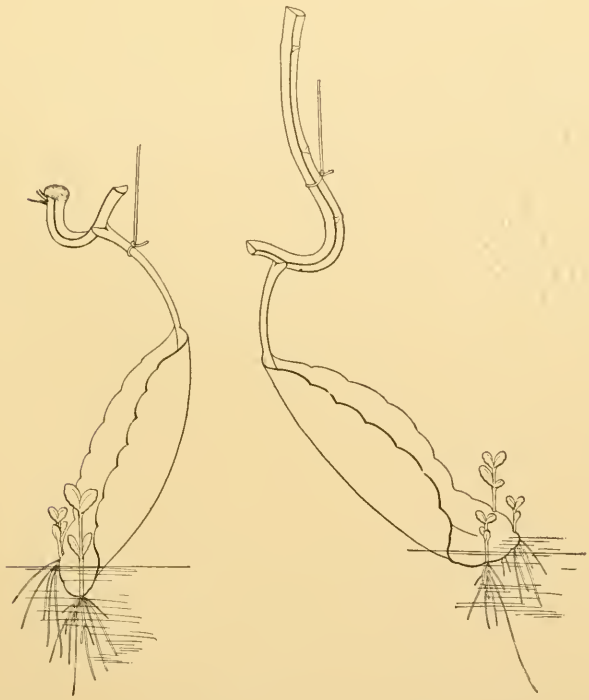

FIG. 87.—See legend of Fig. 86.

where the small piece of stem with a leaf near the base forms considerable callus while the large piece of stem with the leaf at a greater distance has in the same time formed almost no callus (Nov. 30 to Dec. 14). It is also shown in Fig. 87, where a piece of stem with a pair of sister leaves at the apex was split longitudinally. The greater part of one half stem was cut off in one piece. In the latter piece, where the leaf was nearer the base (to the left in Fig. 87), the callus formation at the base of the stem was considerable while the long piece had not yet formed any callus. A small callus was formed later.

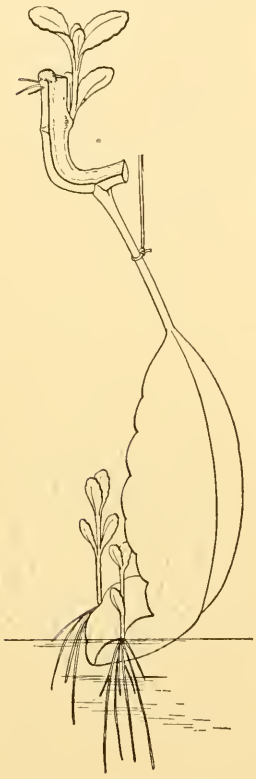

Fig. 88.—Callus formed only on that side of the stem where no shoot is formed.

We have seen that the inhibitory effect of an apical leaf on shoot formation in a stem suspended vertically and upright is greater when the stem is young than when it is old. It is of interest that the callus formation in old stems is also less than in young stems.

All these experiments show the parallelism between the effect of the apical leaf on callus formation and on the inhibition of shoot formation in the basal part of the stem.

Figure 88 shows the inverse relation between callus formation and shoot formation in the basal part of a leaf. The stem in Fig. 88 has a large leaf at the apex which dips into water. In the node below the apex one shoot is formed, while on the other side of the same node none is formed. Only one half of the base of the stem has formed a callus; namely, where there is no shoot; the other half of the base of the stem has formed none, for the reason that here a thickening has occurred in the region of the new shoot and that the material was consumed here which otherwise might have served for callus formation at the base.

The same correlation is shown in Fig. 89. Young stems with a large apical leaf with only one node below the apical one and with the shoot opposite the leaf removed, form no shoots in the

first node below the leaf and only after more than a month's delay a shoot may arise in the axil of the leaf. But instead a vigorous callus is formed at the base of the stem. This result, which is typical, suggests that the greater part of the material sent down by the apical leaf is consumed in callus formation. Had a shoot been formed in the stem this callus formation would have been less.

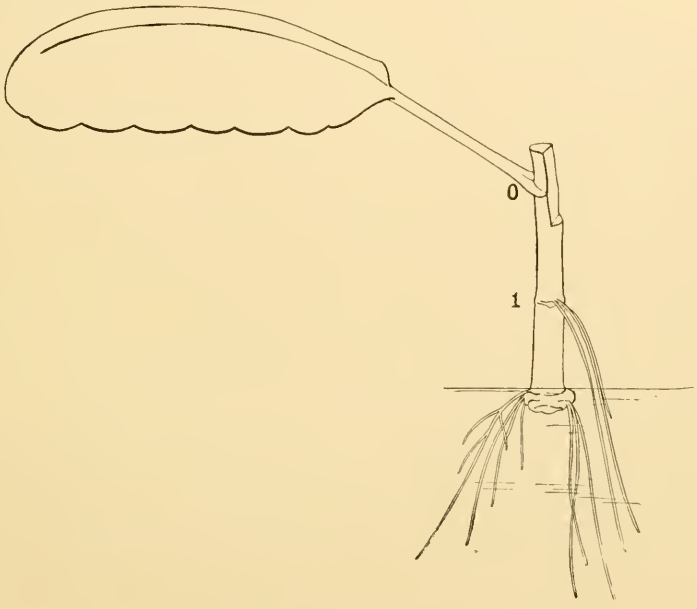

FIG. 89.—Stem with an apical leaf in which shoot formation is suppressed forms large amount of callus. Oct. 16 to Nov. 14, 1923.

The formation of callus may be considered as the result of the growth of certain tissues of the stem under the influence of the descending sap. It is possible that these tissues are also those which give rise to roots and perhaps to the growth in the length and possibly thickness of the stem. We can understand that this growth of the stem might indirectly inhibit the growth of shoots in the same part of the stem, though for the explanation of the mechanism of such an inhibition further experiments will be necessary.

CHAPTER XIV

INHIBITORY EFFECTS OF A SECOND ORDER BY AN APICAL LEAF

Aside from the inhibitory effects of an apical leaf on shoot formation in the direct path of the descending sap there exist other small inhibitory effects outside this path. These latter inhibitory effects outside the path of the descending sap from an apical leaf on shoot formation may be called inhibitory effects of a second order to distinguish them from the inhibitory effects which lie directly in the descending path. These inhibitory effects of the second order show themselves in the fact that in stems with one apical leaf even the shoot formation on the side of the stem opposite to that of the leaf is delayed and comparatively diminished.

Six short stems with one leaf each at the base (Fig. 90) were put with the base of the stem in water. Each stem formed only one shoot at the apex and roots at the base. The experiment lasted from Dec. 7, 1922 to Jan. 5, 1923; the dry weight of the 6 leaves was 2.8998 grams, and the dry weight of all the shoots produced by the stems was 348 milligrams. One gram of leaf produced therefore 120 milligrams of apical shoots.

Simultaneously an experiment was made with 5 short stems, each of which had a leaf at the apex (Fig. 80 in Chapter XII). In this case 2.607 grams of dry weight of apical leaves produced 180 milligrams of shoots in the basal part of the stem, or 1 gram dry weight of apical leaf produced only 68 milligrams of shoots. The difference between the effect of the leaf on shoot production in the ascending and descending sap cannot be attributed to a difference in the dry weight of the stems since the dry weight of the stems in the experiment with the ascending sap (Fig. 90), where more shoots were formed, was only 1.87 grams as against 3.9 grams in the experiment with the descending sap (Fig. 80). The only conclusion is that the descending sap sent out by the leaf formed in this experiment per gram dry weight of leaf only

about one-half of the quantity of shoots that 1 gram of dry weight of leaf formed through the ascending sap.

Now in these experiments no shoots or roots were formed in the leaves themselves. On the basis of the experiments mentioned in Chapter VI, we must conclude that the leaves sent out the greater part of their material into the stem in both experiments and that hence the diminution in the quantity of shoot production by the descending sap must be attributed to the fact that not quite as much of the material sent out by the apical leaf was

FIG. 90.—Shoot formation in stem with basal leaf.

utilized for shoot formation as was utilized in the case of the basal leaf. The stem must have retained in some form a greater part of the material sent out by the apical leaf. That such a retention of material in the stem seems actually to occur to a greater extent in the case of the descending than in the case of the ascending sap is also shown by the fact that the shoot formation in a piece of stem with an apical leaf is delayed and diminished the more, the greater the distance between the place of shoot formation and apical leaf; while no such relation exists in the case of the ascending sap.

Six young stems with one apical leaf each were suspended with their base in water. In this case each of the stems formed one shoot opposite the leaf (Fig. 91). The dry weight of the 6 leaves

was 2.082 grams and the dry weight of the 6 shoots was 274 milligrams. One gram of leaf produced therefore 132 milligrams dry weight of shoots. (The experiment lasted from Nov. 8 to 28.)

Simultaneously 6 stems also with one apical leaf each, but with the anlage of the shoot opposite the leaf cut out (Fig. 92), were

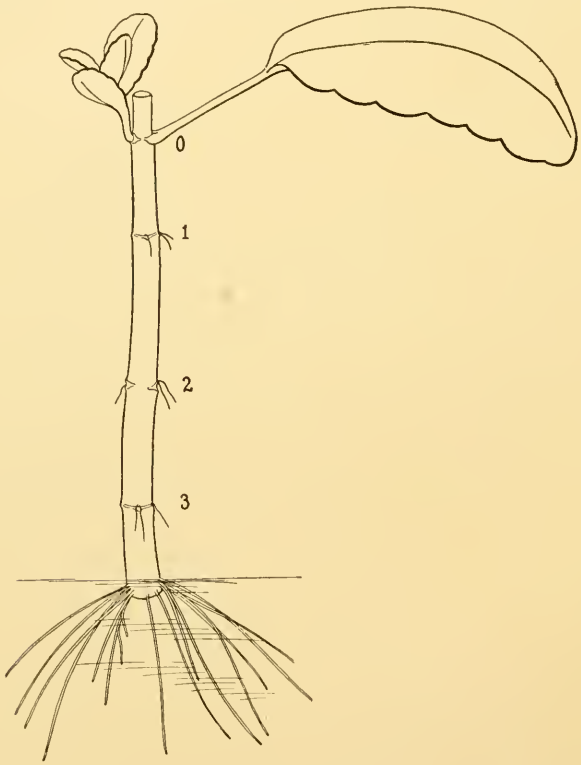

Fig. 91.—Growth of shoot is more rapid in Fig. 91 than in Fig. 92, p. 117.

treated in the same way. In this case each stem formed a shoot in the second node below the leaf but on the side opposite the leaf (Fig. 92). The shoot formation began later and 2.053 grams of dry weight of leaf produced 126 milligrams dry weight of shoots, or 1 gram dry weight of leaf produced 62 milligrams of shoots. Hence the same mass of apical leaf produced a smaller mass of shoots when the sap from the leaf had to travel farther downwards before reaching the anlagen for the shoot. The inference sug-

gested itself that in the latter case some of the material sent out by the leaf was consumed by the stem in the first two internodes, so that less remained available for shoot formation.

The fact that the shoot formation in the basal part of the stem diminishes the more the greater the distance between the apical leaf and the new shoot is strikingly illustrated by the following

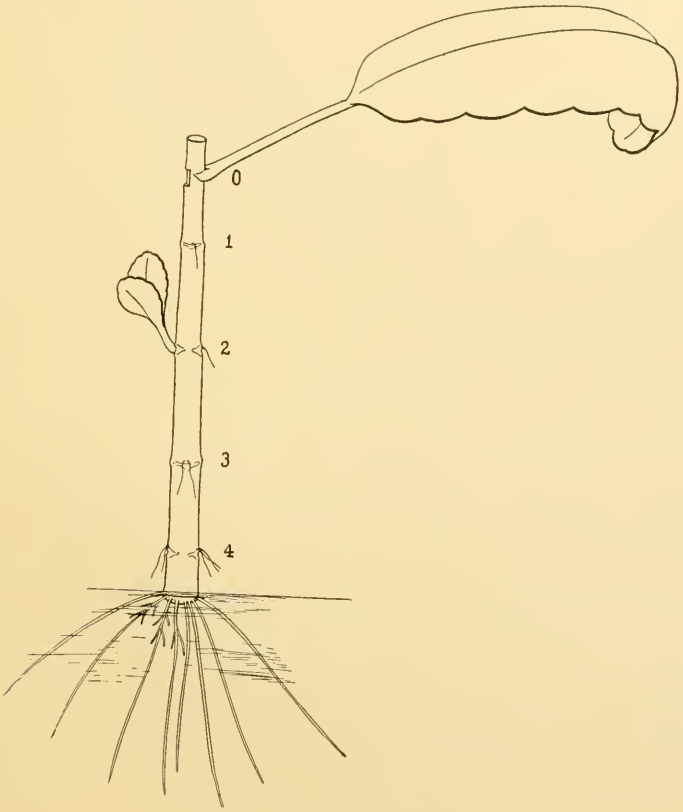

FIG. 92.—See legend of Fig. 91.

experiments (Figs. 93 to 96) which were made simultaneously and lasted from Oct. 2 to 23: In Fig. 93 a stem less than one year old with one apical leaf was split longitudinally and both pieces were suspended in an aquarium with the base of the stems dipping into water. The pieces with an apical leaf formed roots but no shoots, the pieces without apical leaves formed shoots but no roots. (Roots would, however, have developed later.) Six

FIG. 93.—Stem split longitudinally, one half with a leaf at the apex, the other without leaf. The one with a leaf forms roots but no shoots, the half stem without a leaf forms shoots but no roots. Oct. 2 to 23, 1923.

FIG. 94.—Left: Half stem removed with exception of region near the apical leaf opposite which a shoot is formed. Right: Half stem removed with exception of piece at first node below the leaf. No shoot is formed. Leaf at apex. Oct. 2 to 23, 1923.

half stems without leaves, with a total dry weight of 1.557 grams, produced in all 0.033 gram of shoots during the 3 weeks the experiment lasted, *i.e.*, 20 milligrams dry weight of shoots per gram dry weight of stems.

In Fig. 94 (to the left) half of the stem below the most apical node (designated 0) was removed. In this case a shoot was formed opposite the leaf. Six stems were used and the dry weight of the regenerated shoots was 234 milligrams. The dry weight of

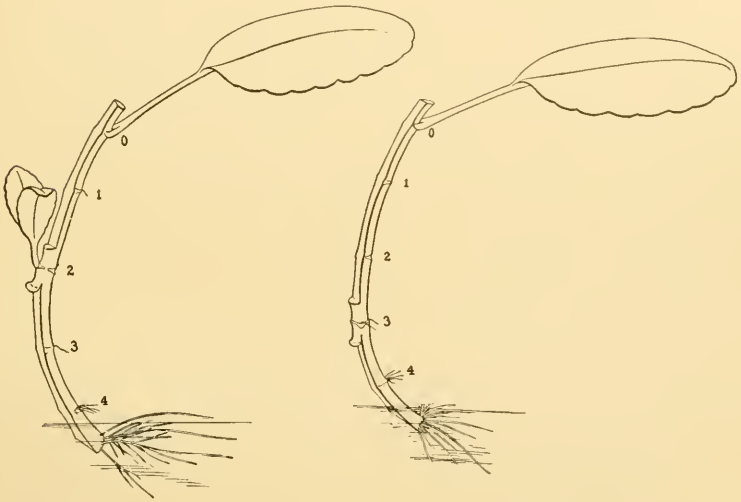

FIG. 95.—Left: Half stem removed with exception of piece in second node where a shoot is formed. Right: Half stem removed with exception of piece near third node below the leaf. No shoot is formed. Leaf at apex. Oct. 2 to 23, 1923.

the 6 apical leaves was 2.183 grams. If we assume that the shoots were formed exclusively from material furnished by the leaf (which may not be entirely correct), 1 gram of leaf produced 107 milligrams of shoots in node 0.

In Fig. 94 (to the right) the stem was split in such a way that the stem near the first node below the leaf (designated as 1) remained intact. In this case no regeneration of shoots occurred, as was to be expected from what has been said, the two buds of the node lying in the path of the sap descending from the leaf.

In Fig. 95 (to the left) the stems were split with the exception of the second node below the leaf (designated as 2), and in this node a shoot grew out. The total dry weight of these shoots was

only 138 milligrams while the total weight of the six apical leaves was 2.465 grams. In this case 1 gram of dry weight of leaves produced only 56 milligrams of shoots.

The third node in Fig. 95 (to the right) produced no shoots as was to be expected, but the fourth node in Fig. 96 produced shoots, the total dry weight of which was only 88 milligrams per 2 451 grams dry weight of leaves. One gram dry weight of leaves produced only 36 milligrams dry weight of shoots.

Fig. 96.—Half stem removed with exception of region near fourth node where shoot is formed. Leaf at apex. Oct. 2 to 23, 1923.

If we make allowance for the possibility that the ascending sap from the stem furnished part of the material of these shoots, calculating this effect from the fact that 1 gram dry weight of stem (without leaves) produced 20 milligrams dry weight of shoots, the absolute figures given for the material furnished by the leaf for shoot formation will be slightly diminished, but the relative values will not be materially altered, and the chief result will remain the same; namely, that the material furnished by an

apical leaf for shoot formation will be the less, the farther the sap has to travel down the leaf, the ratio being as follows:

One gram of leaf produces 107 milligrams dry weight of shoots in node 0.
One gram of leaf produces 56 milligrams dry weight of shoots in node 2.
One gram of leaf produces 36 milligrams dry weight of shoots in node 4.

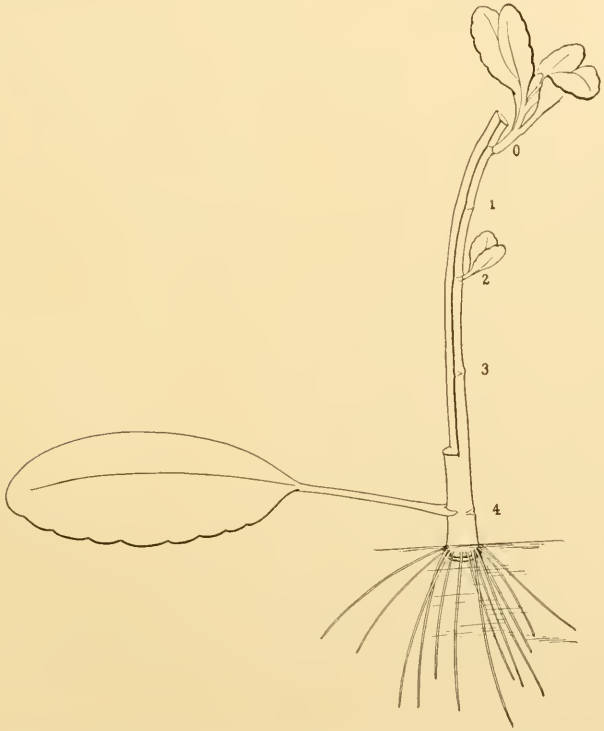

FIG. 97.—Half stem with leaf at base. Nov. 12 to Dec. 5, 1923.

The simplest explanation for this difference seems to be that in young stems, which are still in the process of vigorous growth, material of the descending sap is retained or consumed in the stem (for the growth of the stem?) and that for this reason the material available for shoot formation by the descending sap from the apical leaf diminishes with increasing distance of the seat of shoot formation from the leaf.

When the reverse experiment is made, namely an investigation of the influence of a basal leaf on shoot formation at the apex, no such diminution with the distance of the apex from the leaf is observed; possibly, perhaps, the contrary occurs; namely, that the mass of shoot formation at the apex increases with the distance of the apex from the basal leaf. Such experiments are

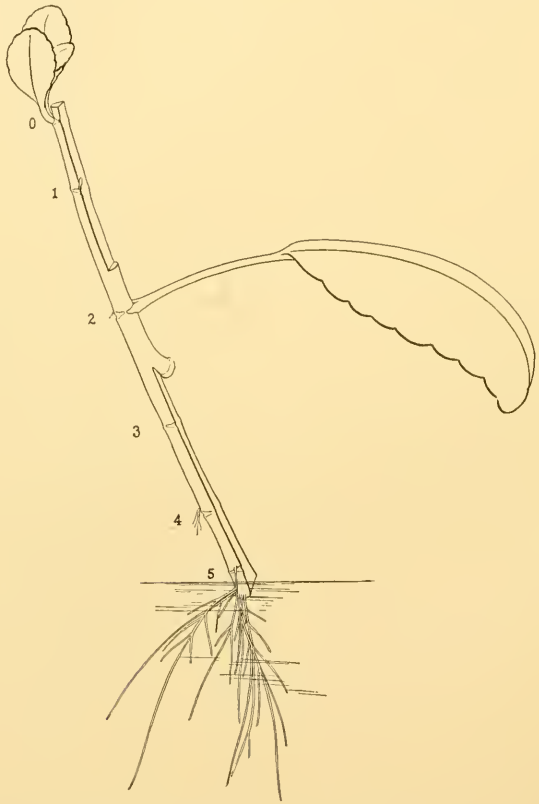

FIG. 98.—Half stem with leaf in middle. Oct. 15 to Nov. 7, 1923.

illustrated in Figs. 97 to 99. The stems were split longitudinally with the exception of the region near one node at which a leaf was left. This node was in turn the fourth (Fig. 97), the second (Fig. 98), and the apical (Fig. 99), thus corresponding to the preceding experiment. The results are expressed in Table XXIV.

The dry weight of the stems was in succession, 3.209, 2.610, and 2.897 grams. The duration of the experiment was 23 days.

TABLE XXIV

One gram dry weight of leaf in fourth node produces 147 milligrams dry weight of apical shoots.

One gram dry weight of leaf in second node produces 111 milligrams dry weight of apical shoots.

One gram dry weight of leaf in apical node produces 83 milligrams dry weight of apical shoots.

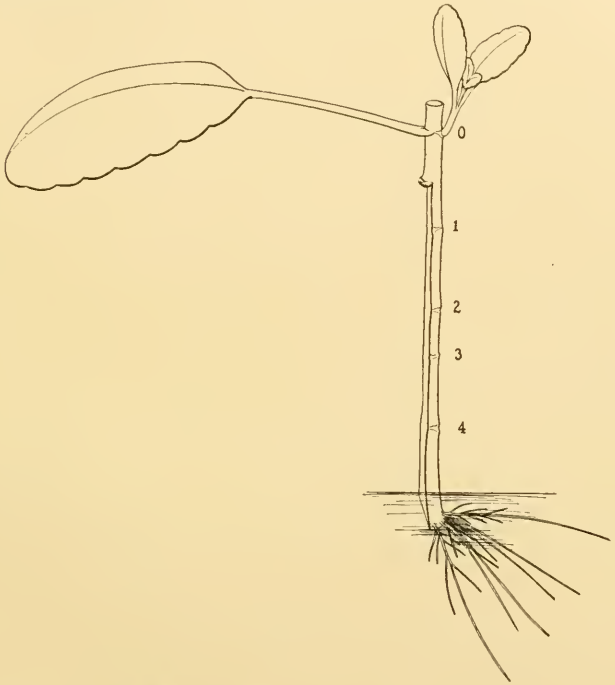

FIG. 99.—Half stem with leaf at apex. Nov. 12 to Dec. 5, 1923.

While the absolute figures would be slightly altered, the main result would remain, that the contribution of a basal leaf to shoot formation at the apex does not diminish with the distance of the leaf from the apex, but rather increases.

We would assume that these inhibitory effects of a second order are also caused by the descending sap from an apical leaf; but whereas the inhibitory effect in the direct path of the descending sap is almost absolute the inhibitory effect outside the path shows itself only in a delay and in a slight diminution of the shoot formation.

CHAPTER XV

THE INHIBITORY EFFECT OF AN APICAL LEAF ON SHOOT FORMATION IN A STEM SUSPENDED HORIZONTALLY

The account of the inhibitory effects of an apical leaf on the shoot formation in young stems given in Chapters XII and XIV is incomplete since it was concerned only with phenomena of inhibition in a stem suspended vertically and upright. When a stem is suspended horizontally and there is only one leaf at the

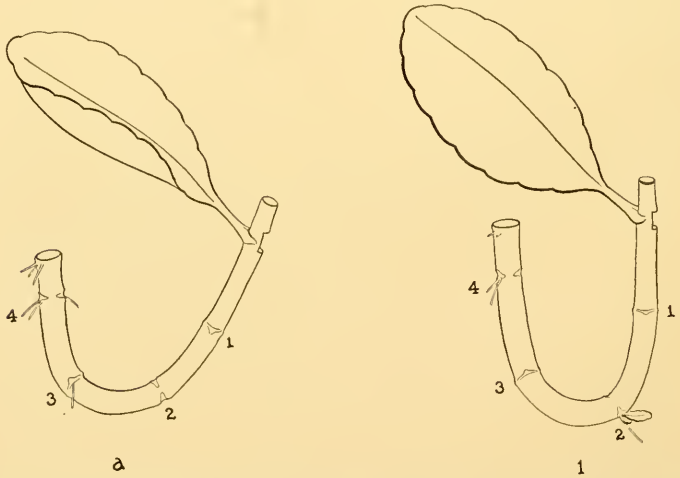

Fig. 100.—Stems originally horizontal but with leaf on upper side. No shoot or shoots formed on lower side in second node basally from leaf. Mar. 16 to Apr. 3, 1923.

apex of the stem the phenomena of inhibition vary according as to whether the leaf is on the upper or the lower side of the stem. This is illustrated by the Figs. 100 and 101. The four stems used in these experiments were originally perfectly straight and were suspended in moist air. The two stems in Fig. 100 had the apical leaf on the upper side of the stem and the two stems in Fig. 101 had the apical leaf on the under side. The anlagen for the apical

shoot opposite the leaf was cut out in all four stems. In all these stems the apical leaf was rather large. The stems bent geotropically so that during the experiment, which lasted from Mar. 16 to Apr. 3, 1923, they assumed the shape of a U, the concavity being on the upper side.

The stems in which the apical leaf was on the lower side (Fig. 101) formed a shoot in the second node basally from the leaf and on the upper side of the stem. This was to be expected since the upper side of the stem is opposite to that of the leaf. This is what happens also when the stem is suspended vertically. But some of the stems formed shoots also or exclusively in the first

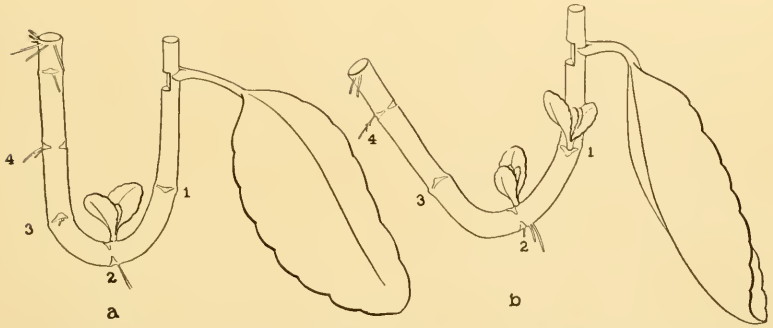

FIG. 101.—Stems originally horizontal with leaf on lower side. Shoot formed in second node on upper side or in both first and second node on upper side. Mar. 16 to Apr. 3, 1923.

node basally from the leaf, and this is different from what would have happened if the stem had been suspended vertically, since in the latter case *no* shoots would have been formed in the first node, provided the leaf was not too small and the stems sufficiently young.

When the apical leaf is on the upper side of the stem suspended horizontally (Fig. 100), the results are still more striking. In this case the stem sometimes forms no shoot at all, or if it forms a shoot its appearance is delayed for some time. The shoot is formed in the second node basally from the leaf on the lower side of the stem (which is opposite to that where the leaf is attached).

It is not difficult to understand these results on the basis of the assumption that there are two channels for the flow of sap in the stem, one through preformed vessels and one through the

tissue spaces, that is the gaps between the cells or the tissues. As had been stated previously, it is chiefly the tissue sap flowing in these latter channels which is influenced by gravity collecting on the lower side of a stem or a leaf; while the sap flowing in the regular vessels is less or not at all influenced by gravity. The vessels for the descending sap from an apical leaf lie on the side where the leaf is attached. When we remember that the descending sap from a leaf inhibits shoot formation in its path we can understand the results in Figs. 100 and 101.

In Fig. 101 the tissue sap will go to the lower side of the stem and on this side flows also the descending sap from the leaf following the regular vessels, since the leaf is on the lower side of the stem. Hence we have no shoot formation on the lower side of the two stems in Fig. 101, but we notice root formation on the lower side and in both cases in the second node and in the fourth node basally from the leaf. These are the nodes in which the inhibition of shoot formation is most complete. Old stems with one apical leaf suspended vertically can form shoots in the first node below the leaf; in the second node only on the side of the stem opposite the leaf, but not on the side where the leaf is attached. The anlagen for shoots in the second and fourth nodes, on that side where the apical leaf is, lie in the middle of the path of the sap flow descending from the leaf and here the inhibition of shoot formation is most pronounced. As Fig. 101 shows, in the second and fourth nodes basally from the leaf and in the same node as the leaf the formation of roots is most favored. It is natural to correlate these two facts.

The question then remains why shoots can arise more easily in the first node basally from the leaf when the leaf is on the lower side of a stem placed horizontally than when the stem is suspended upright and vertically. This is intelligible with the aid of Fig. 70 (Chapter XII) which shows the path of the sap descending from a leaf. The two anlagen for shoots in the first node basally from the leaf lie at the outer edge of the sap flow from the apical leaf. When the stem is suspended vertically and upright the two anlagen for shoots in the first node below the leaf will be bathed in tissue sap escaping from the vessels containing the descending current from the apical leaf. When the stem is placed horizontally some of this sap will sink, thus allowing some of the buds in the first node below the leaf to escape the full consequence of inhibitory effect.

This explanation is supported by the experiments represented in Fig. 100 where the apical leaf of the stems placed horizontally was above. In this case we have inhibition above and below. On the upper (concave) side of the stem shoot formation is inhibited because this is the side where the descending sap flows from the apical leaf. On the lower side it is inhibited because there the tissue sap collects. This inhibition of the shoot formation by the tissue sap is, however, less complete than that by the sap flow in the regular vessels carrying the descending sap.

Some new light is thrown upon the inhibiting effect of an apical leaf on shoot formation in the basal part of the stem by the

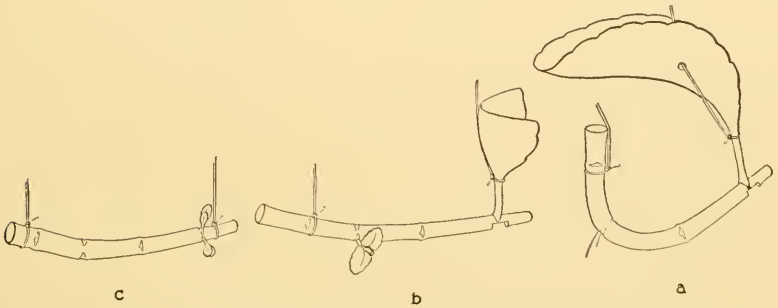

FIG. 102.—Shoot formation in stem *a*, with whole leaf at apex has not yet begun; in stem *b*, with half leaf, a shoot formed in the second node below; also shoots in stem *c*, without leaf. Apr. 25 to May 10, 1923.

fact that the shoot formation on the side of the stem opposite from that of the leaf is at first inhibited and later increased in proportion to the mass of the leaf.

Young stems of *Bryophyllum* each possessing 4 nodes were suspended horizontally in air. One set of stems had a large leaf at the apex on the upper side of the stem (Fig. 102, *a*) and a second set (102, *b*) had a leaf of about one-third the size of the leaf in *a*, also on the upper side of the stem; while a third set of stems (Fig. 102, *c*) had no leaf. In *a* and *b* the anlagen for shoots opposite the leaf had been cut out. The drawing was made on the fifteenth day. None of the stems with a large leaf (*a*, Fig. 102) had formed a shoot, only a root on the lower side in the second node; three among four stems with reduced leaf (*b*, Fig. 102) had each formed a shoot on the lower side of the stem (opposite from that of the leaf), and three among four stems without a leaf had formed apical shoots. Hence a large leaf at the apex at

first retards shoot formation more than a small leaf; a stem without a leaf is altogether free from this inhibition to shoot formation.

Figure 103 was drawn 13 days later. In the meantime the result was completely changed. The stems with a whole leaf

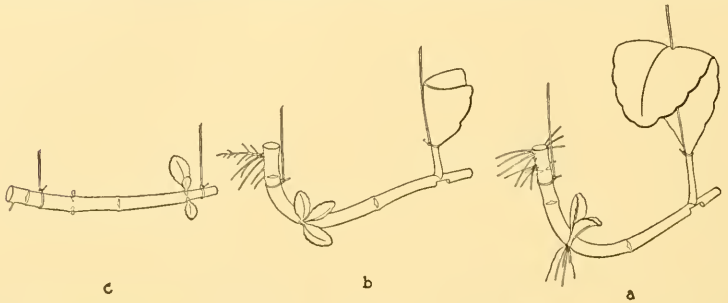

FIG. 103.—Same stems as in Fig. 102, 13 days later, May 23. *a* has now formed a larger shoot than *c*.

a had not only formed a shoot (in the second node basally from the leaf and on the opposite side of the stem) but this shoot had grown so rapidly that it at least equalled the shoot in stem *b*, and probably exceeded in mass the two shoots in stem *c*.

Six days later, that is, on the thirty-fourth day of the experiment, the condition was that given in Fig. 104, namely the mass of

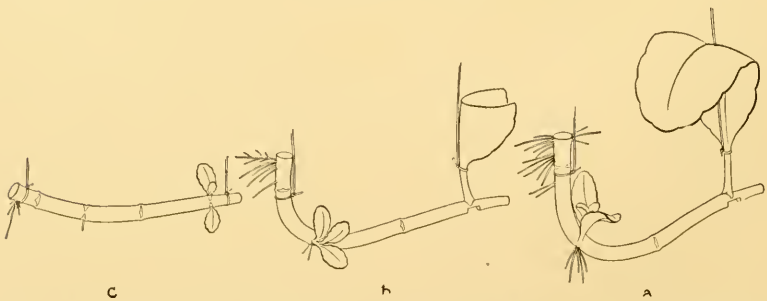

FIG. 104.—Same stems as in Fig. 102, 19 days later, May 29. Shoot in *a* greater than in *b*.

shoots formed in the three sets of stems varied with the mass of the apical leaf. The stems *a* with the largest leaf had the largest shoot, while the stem without leaf had the smallest shoots. The inhibitory effect of the apical leaf existed therefore only at the beginning of the experiment; when the experiment continued long enough the mass law was established again. This was not only

observed in the stems drawn in Figs. 102 to 104, but was a general result. It is incompatible with the idea that the initial inhibitory effect of the apical leaf on the shoot formation in stem *a* was due to an inhibitory hormone, since it is contradicted by the ultimate result that the shoot in stem *a* exceeds in mass those of stems *b* and *c*.

Similar phenomena of inhibition (of the second order) are observed when the leaf is below. Young stems (as in Fig. 105, *a* and *b*) were suspended horizontally, the leaves dipping into water, *b* had a large leaf, the leaf in *a* was considerably

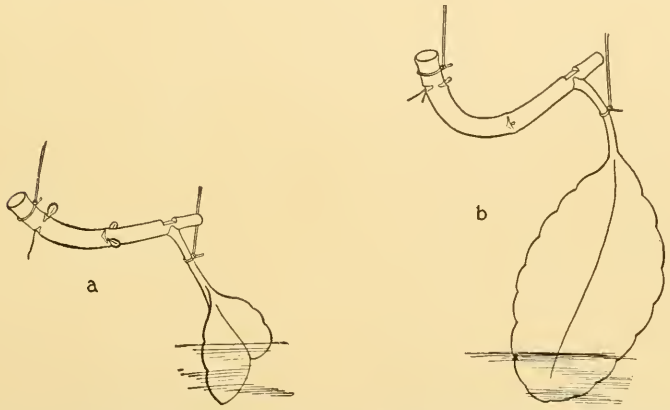

FIG. 105.—Leaf on lower side of stem. Stem *b*, with whole leaf, has not yet commenced to form a shoot, while the stem with a smaller piece of leaf has commenced to form shoots. May 16 to 24, 1923.

reduced. The shoot formation commenced a little earlier in *a* than in *b*. The drawing was made on the eighth day. Five days later, that is on the thirteenth day of the experiment, the shoot in the stem with a large leaf exceeded in mass the shoots in the stem with a small leaf (Fig. 106). On the twenty-third day the experiment was interrupted and the dry weight of the leaves, shoots, and stems was determined. Two sets each with 5 stems were used for the experiment. The dry weight of the 5 whole leaves (*b* and b_1 in Figs. 105 and 106) was 1.258 grams and their stems produced in all 370 milligrams of shoots; or 1 gram of leaves produced 293 milligrams of shoots. The total dry weight of the 5 reduced leaves (*a* and a_1 in Figs. 105 and 106) was 0.328 gram and the stems produced only 111 milligrams of shoots; or 1 gram dry weight of leaf produced 337 milligrams dry weight of shoots,

which is not far from what we should expect according to the mass law.

If we inquire how it happens that at first the apical leaf, if it is sufficiently large, inhibits even transitorily the shoot formation on the side of the stem opposite to that of the leaf, we can only suggest that it might be connected with a growth of tissues in the stem. Should the inhibition be correlated with the fact that the growth giving rise to geotropic curvature of the stem precedes that of the regenerative processes? The reader will notice that this curvature increases with the mass of the apical leaf and that

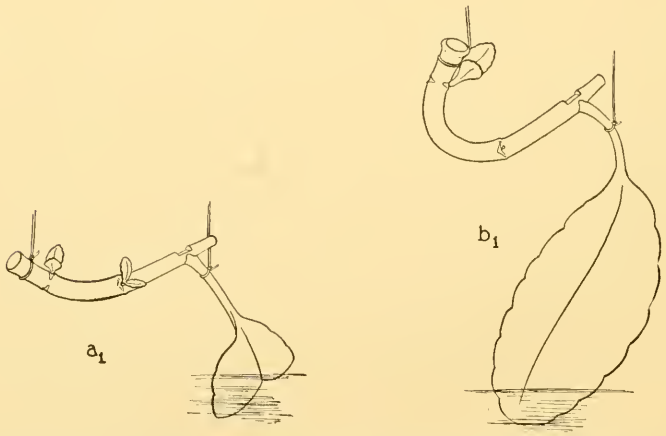

FIG. 106.—Same stems as in Fig. 105, 5 days later, May 29. In stem b_1, with whole leaf, a shoot on the upper side has grown rapidly, being larger than the shoots in stem a_1.

the difference in curvature is more marked at first than later. It is not impossible that this growth in the stem might cause indirectly an inhibition of shoot formation. The main fact is that these inhibitory effects of the second order exist only at the beginning.

If we try to explain all of these phenomena of inhibition of the first and second order in harmony with the theory of the polar character of regeneration at which we arrived, we must assume that the growth of the stem caused by the descending sap from a leaf leads to a retardation or complete inhibition of the growth of shoots in the basal part of the stem. In the direct path this inhibition is most complete (inhibition of the first order). Outside the path the inhibition is less but still noticeable in the form

of a temporary retardation of shoot formation (inhibition of the second order). When the apical leaf is reduced in size the growth of the stem inside the path of the descending current will be less and hence the inhibitory effect on shoot formation (both of the first and second order) will also be less. This assumption explains the phenomena of inhibition qualitatively as well as the hormone hypothesis and has the advantage of being in harmony with the theory of polarity. But how the growth in the stem inhibits the growth of shoots remains unexplained.

CHAPTER XVI

THE GROWTH OF THE AXILLARY SHOOTS

We have seen that a completely defoliated stem forms a pair of shoots in its most apical node. Each of the two anlagen for these shoots was located in the axil of a leaf. When two leaves

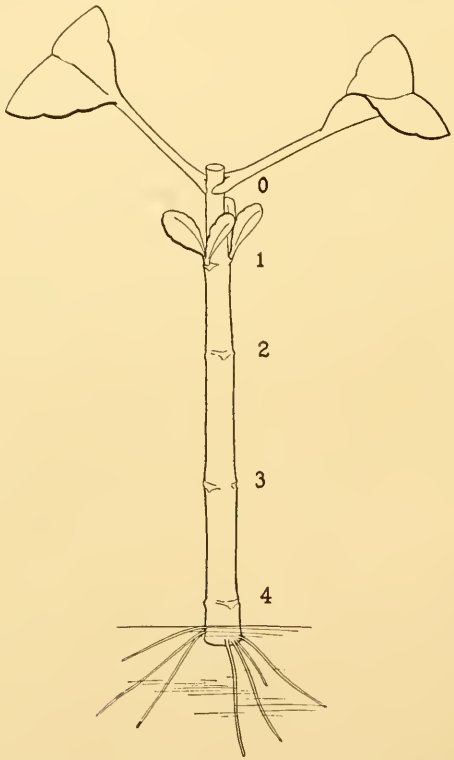

FIG. 107.—Young stem with two apical leaves reduced in size. Shoots formed in the first node below the leaves, but not in the axil of the apical leaves. Nov. 28 to Dec. 18, 1923.

are left at the apex of a stem otherwise defoliated and the piece of stem is old shoots are formed not in the axils of the two apical leaves but in the node below. If the stem is young and if it is defoliated with the exception of two large leaves in the most

apical nodes, the formation of shoots in the whole stem is inhibited, but if the size of the two leaves is sufficiently reduced the stem may form two shoots (Fig. 107); the shoots, however, do not grow out from the axil of the leaves but in the node below. The inhibitory effect of the leaf on the growth of its axillary bud is therefore greater than the inhibitory effect on the growth of the anlagen below; but it is, perhaps, not greater than the inhibitory effect of an apical leaf on the formation of a shoot in the second node below the leaf and on the same side of the stem as the leaf. The question is, under which conditions is it possible to cause the growth of a shoot in the axil of a leaf without removing the latter? This can be done by selecting a large leaf and cutting off

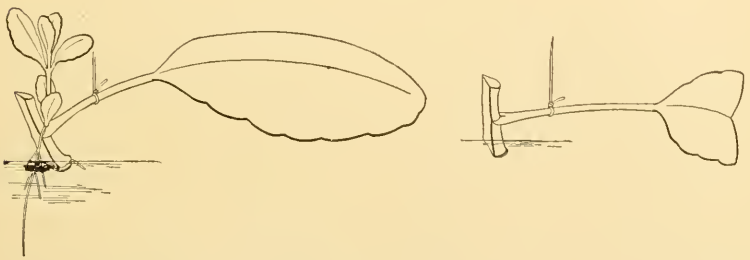

FIG. 108.—Axillary shoots are formed when the stem is short and the leaf large, but not when the leaf is reduced in size. Nov. 15 to Dec. 12, 1923.

the whole stem with the exception of a small piece, with no other bud except that in the axil of the leaf. When the leaf is small, the chance of a shoot growing out from the axil of the leaf is smaller than when the leaf is large. In the experiment in Fig. 108 a small piece of stem with one node and a pair of leaves was split longitudinally. One leaf remained intact, the other was reduced in size. Only in the axil of the large leaf was a shoot formed. This experiment yields the same result when the leaves dip into water and when the stems are in air.

In this case the inhibition of the growth of shoots can be neither due to an inhibitory hormone sent out by the leaf nor to any increase of growth in the stem due to the leaf; since in either case the inhibitory effect of the leaf on the growth of the axillary shoot should increase with the size of the leaf.

When the stem is large the chances of the axillary bud growing out are also smaller than when the stem is small.

In such experiments as represented in Fig. 108, one cannot count with certainty on the growth of the axillary bud. This is, however, possible if the petiole of the leaf, where it joins the stem, is reduced in size. It does not matter whether the lateral parts of the petiole at the node are cut off as in Fig. 109, or whether a piece is cut from the center of the petiole, where it joins the node

Fig. 109. Fig. 110.

Fig. 109.—An axillary shoot is formed when the petiole of the leaf at juncture with stem is reduced laterally. Nov. 23 to Dec. 15, 1923.

Fig. 110.—An axillary shoot is formed when a hole is made through the petiole in the center where it joins the stem. Nov. 25 to Dec. 15, 1923.

of the stem, as in Fig. 110. In either case, a shoot will grow out from the axillary bud of the leaf—provided the piece of half stem is small as in Figs. 109 and 110.

Is it lack of oxygen which prevents the axillary bud of a leaf from growing into a stem or is there some other cause? The writer is not in a position to answer this question at present.

It may be permitted to mention here briefly some asymmetries in shoot formation in the first and second node apically from the leaf. This phenomenon was most clearly observed in potted stems the apex of which was cut off and which were defoliated, with the exception of one leaf left in the first or second node below the most apical node of the stem (Figs. 111 and 112). When the leaf was in the first below the most apical node, the two leaves of the new shoots growing out in the apical node were no longer identical in size (as they usually are) but the new

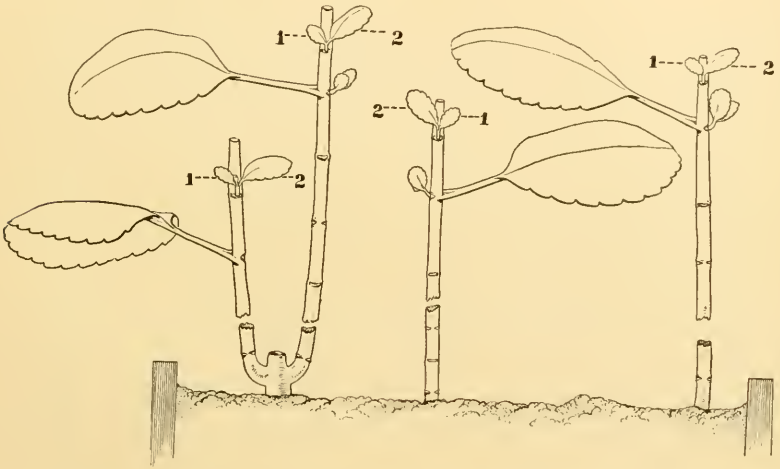

FIG. 111.—Old leaf left at first node below the apical node. The two leaves, 1 and 2, of the new shoot in the apical node (which are normally of equal size) show a constant difference, leaf 1 (on the side where the old leaf is) being smaller than leaf 2 (on the opposite side).

leaflet on that side of the stem where the old leaf was, was always smaller, sometimes completely suppressed. The smaller leaflet is marked 1 and the larger leaflet is marked 2, in Fig. 111. When the old leaf is in the second node below the most apical node, the second pair of leaflets in the new shoot shows this asymmetry (Fig. 112). These asymmetries disappear when the old leaf is so small that the sap sent out by the leaf is negligible compared with the sap in the stem. It is possible that these asymmetries are the result of a crossing of the vessels for the ascending sap in a node, whereby the new leaflet on the opposite side from the large leaf receives more sap than the other.

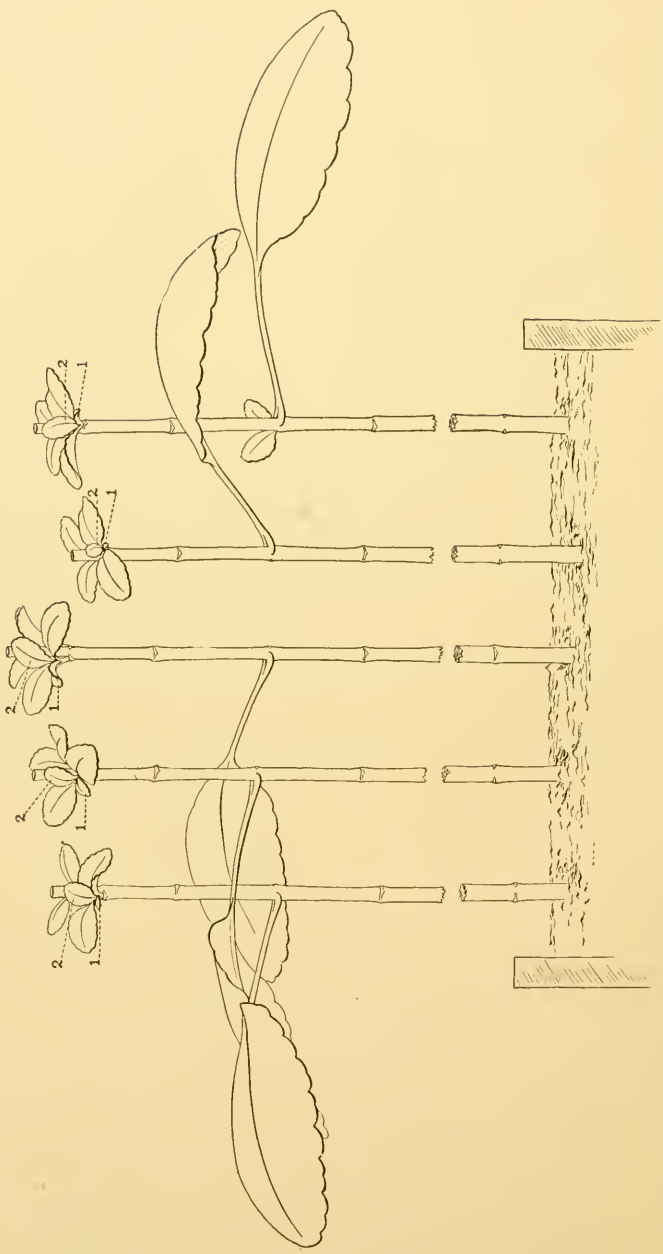

FIG. 112.—In the second node above the old leaf the asymmetry effect is shown in the second pair of leaves of the new shoot which are no longer of equal size, leaf 1, which is above the old leaf, being a little smaller than the symmetrical leaf 2. Duration of experiment, Oct. 17 to Nov. 13.

CHAPTER XVII

SOME PRELIMINARY EXPERIMENTS ON THE PATH OF THE ASCENDING AND DESCENDING SAP IN THE STEM OF *BRYOPHYLLUM*

The experiments on polarity have led to the idea that the polar character of regeneration in the stem of *Bryophyllum* is not due to the chemical differences between the ascending and descending sap which in all probability exists, but to the fact that the anlagen for shoots and roots are located in different tissues and that under ordinary conditions, the ascending sap reaches primarily the anlagen for shoots while the descending sap reaches primarily the anlagen for roots. The inhibition of shoot formation by the descending sap is apparently a secondary phenomenon caused perhaps by the fact that the descending sap reaches primarily the tissues for root formation and for the growth of the stem and that this growth in some way not yet cleared up inhibits temporarily or permanently the growth of neighboring shoots.

It was natural then to attempt to find out whether the ascending and descending sap flow in different channels. This might be possible of demonstration, provided, first, that the channels for the descending and ascending sap lie in different concentrical layers in the stem, the one more peripheral than the other; and second, that after removal of one type of channel no restitution of the channels occurs by regeneration.

In one set of experiments (Fig. 113) in pieces of stem split longitudinally and consisting of two nodes and one leaf at the base, the wood was removed in the upper part, between *b* and *a*. Such stems still formed apical shoots, proving that the ascending sap can reach the anlagen for shoot formation through the cortical layers alone. In other experiments in pieces of split stem the lower part of the wood between *b* and *c* (Fig. 114) was removed. Such stems were still capable of forming roots at the base and also shoots in the basal part. Hence the ascending and descending sap can reach both the anlagen for shoots and roots through the

cortical layers alone, although the rate of growth of both roots and shoots is considerably retarded when part of the wood is removed.

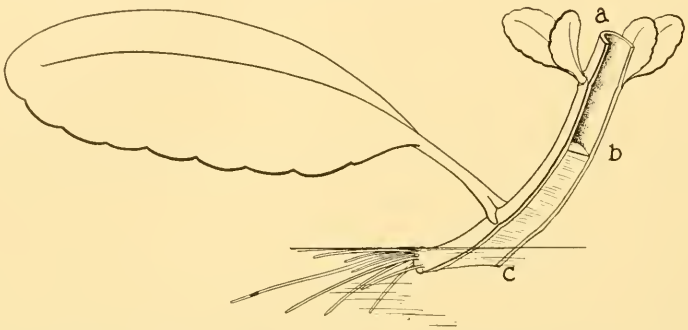

FIG. 113.—Proof that shoots can be formed by cortex alone. Oct. 16 to Nov. 8, 1923.

The basal end of the cortex can also form a callus if it is deprived of the wood, though this callus formation is slight at the best. The experiments lasted from Oct. 16 to Nov. 8, 1923.

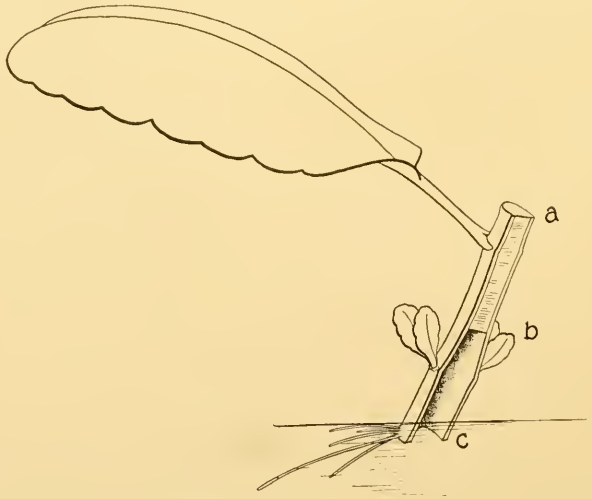

FIG. 114.—Proof that roots can be formed by cortex alone. Oct. 16 to Nov. 8, 1923.

For the next experiment stems such as in Fig. 115, *a*, were used. They possessed three nodes and a pair of large leaves in the middle nodes. A ring of cortex was removed from a piece of

the stem in the internode above as well as below the leaf (*a*, Fig. 115). Regeneration occurred quantitatively as if no cortex had been removed from the stem. The most apical node formed shoots while the shoot formation in the most basal node was inhibited. Roots were formed only at the base. That the shoot formation at the apex of *a* was due to the ascending sap sent out by the leaf is shown by the pieces *b* and *c* (Fig. 115), which had no

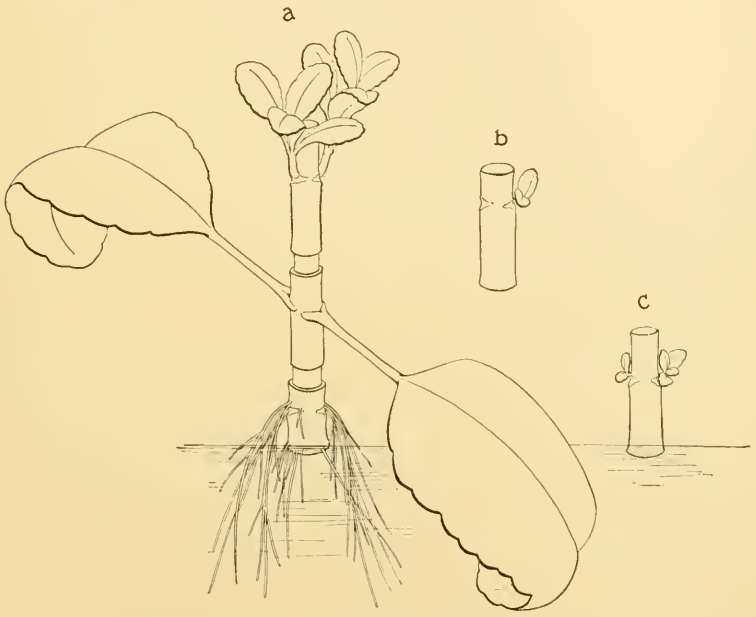

FIG. 115.—Proof that both ascending and descending sap are conducted when parts of cortex are removed. Oct. 26 to Nov. 15, 1923.

leaves; *b* was suspended in air, and *c* dipped into water; both had formed only tiny shoots. That the root formation at the base of *a* was due to the descending sap from the leaf was also shown by the fact that the pieces *b* and *c* had formed no roots. These experiments, all of which turned out in the same way, show that the ascending and descending sap from the leaf can move through the stem even where the cortex is removed. Only the rate of regeneration is retarded in this case. The experiment lasted from Oct. 26 to Nov. 15, 1923.

CHAPTER XVIII

THE FORMATION OF FLOWERS IN *BRYOPHYLLUM*

The plants growing in the open in Bermuda differ in two respects from the plants raised in my greenhouse in New York. The Bermuda plants growing in the open have flowers in February or March, and furthermore they form a considerable amount of a purplish pigment (anthocyanin?). Only in shaded places did I find plants in Bermuda which had no flowers and little or no purplish pigment. They were plants which, if my memory does not deceive me, were only exceptionally or not at all reached by direct sunlight.

The plants in my greenhouse have, with a few exceptions, produced no flowers and little purplish pigment. The few plants which produced flowers were in that part of the greenhouse which had the strongest light. It is not impossible that those few plants which produced flowers had been illuminated for short periods by direct sunlight which reached them through the open transom, without the rays of light having been filtered through the glass plates of the roof of the aquarium. It is, however, quite probable that the failure of my plants to produce flowers was connected with the comparative lack of light, and possibly with the lack of ultraviolet light.

Sachs published a paper in 1886[1] in which he tried to prove that ultraviolet light is necessary to produce flowers. He had shown in previous papers that the production of flowers can occur in the apex of *Tropaeolum majus* even if this apex is kept in the dark, provided the leaves of the plant are exposed to sufficiently strong light. This observation formed the basis of his hypothesis of specific or organ-forming substances which are transported in the sap sent out by the leaf.

[1] Sachs, J.: *Arbeiten d. bot. Inst. in Würzburg*, vol. iii, 372, Leipzig, 1888.

CHAPTER XIX

CONCLUDING REMARKS

It is the purpose of this little volume to show that a simple mass relation can be used as a guide through the bewildering maze of the phenomena of regeneration. This mass relation states that equal masses of isolated sister leaves produce, under equal conditions of illumination, temperature, etc., approximately equal masses of shoots and roots in equal time. With this relation it was possible to explain why isolation of a leaf leads to regeneration; namely, because the material available in the leaf for growth flows normally into the stem where it is used for the growth of roots, shoots, and of the stem itself. When a piece of stem inhibits the regeneration in a leaf the stem gains in dry weight to an amount about equal to the diminution in the dry weight of shoots and roots in the leaf due to the stem. It was shown that this mass relation holds also for the formation of roots and shoots in an isolated piece of stem. Equal masses of stems produce under equal conditions of illumination, temperature, etc., approximately equal masses of roots and shoots in equal time.

In both cases a second physiological factor was revealed which must be considered; namely, that the flow of sap in a leaf or stem is secondarily directed towards that part of a leaf or stem where the more rapid growth of shoots or roots occurs. This explains why only some but not all of the anlagen for roots or shoots in a leaf or stem will persist in growing out; while the growth of the others will stop. This again would have remained merely a hypothesis had it not been possible to prove it quantitatively on the basis of the mass relation.

A further complication arises in the fact of the polar character of the regeneration of roots and shoots in the stem of *Bryophyllum*. Two possibilities presented themselves for the explanation of this phenomenon: the polar character of the regeneration in the stem is either due to a difference in the chemical character of the ascending and descending sap or to a difference in the nature of the cells or anlagen which are primarily reached by the ascending and descending sap. It was possible to decide between the two hypotheses by using the mass relation, inasmuch as it could

be shown that the shoots and roots formed in a piece of stem, increase with the mass of the leaf attached to the stem. Since the sap in the leaf forms shoots and roots in the same notch when the anlagen for these two organs exist closely together, the fact that shoots are formed at one and roots at the other end of a stem indicates that the ascending and descending sap of a leaf reach primarily different anlagen, and this conclusion was corroborated by two groups of facts; first, by directing with the aid of gravity the ascending sap in a stem to the tissues capable of root formation roots were produced in abundance by the ascending sap; and second, the mass of shoots produced in the basal part of the stem increased with the mass of an apical leaf. These facts seem to eliminate the idea that the polar character of regeneration is due to any chemical differences between the ascending and descending sap although such differences exist.

The fact that certain mysterious substances like the "vitamines" or "hormones" may accelerate the rate of growth of an organ and consequently perhaps also the rate of regeneration, is neither in contradiction with the mass relation nor with its application to phenomena of regeneration. The sap sent out by a leaf contains probably all the substances needed for growth, inclusive also of "vitamines" and "hormones."

It is quite probable that the principle of mass relation can serve as a guide in phenomena of regeneration in other organisms than *Bryophyllum*. In order to apply the principle we must be able to measure with a certain degree of accuracy the quantity of the material available for the synthetic processes underlying regeneration. This is possible in plants where the leaf is the main organ for the production of this material and where the light is the main source of energy for its production. It is also possible where we deal with definite quantities of stored material, as *e.g.*, in a potato, and where therefore the quantity of the mass of material available for regeneration can be varied at will. Unfortunately it is not easy to control the mass of available material for regeneration in animals. The material from which new organs are regenerated in animals must be furnished either by the food taken up or by the hydrolysis of material in the cells of the animal. All attempts to arrive at a rationalistic theory of regeneration in animals will have to rely on the use of organisms where the mass of material available for regeneration can be controlled as easily as in *Bryophyllum*.

It has always been noticed that gravity has a strong influence on the formation of organs in plants, and Sachs especially has emphasized this fact. A simple explanation of this influence has been offered in this volume; namely, that it is primarily due to a collection of tissue sap in the lower parts of a leaf or a stem placed horizontally. This causes a perhaps slight acceleration of growth which causes secondarily a flow of sap to these more rapidly growing tissues, whereby the growth of tissues on the upper side of the plant organs becomes impossible.

It is a rather striking fact that in contrast with the wide influence of gravity in the arrangement of organs in plants, similar effects in animals are rare. The writer noticed an influence of gravity on the regeneration of organs in a hydroid, *Antennularia antennina*,[1] but this is a great exception. I have often wondered which difference in the structure of plants and animals might be responsible for this difference in the influence of gravity on the formation of organs. If the view expressed in the pages of this book be correct, that this influence of gravity depends upon the collection of tissue sap in the lowest parts of a stem or a leaf, it would seem to follow that such a collection might be more rare in animals than in plants. A collection of tissue sap which is capable of following gravity is known in animals under the pathological condition of edema, but seems to exist only exceptionally in normal animals. Where this is the case the conditions for an influence of gravity on the arrangement of organs might exist.

Certain points in the regeneration of *Bryophyllum* were not touched upon in this little volume since they are not connected with the mass relation, as *e.g.* why it is that the anlagen for shoots are found only in definite points of the leaf of *Bryophyllum*, namely, in the notches; and why in a stem the anlagen for shoot formation exists only in definite spots of the node (in the center of the axil of a leaf) and not in the internode, while anlagen for roots exist throughout certain layers of the cortex of the stem.

It also still remains to be explained that the new shoots regenerated have always the hereditary character of *Bryophyllum* and no other. While these two problems remain unsolved for the present, there is no reason to suppose that they are due to any other than purely physico-chemical agencies.

[1] Loeb, J.: "*Untersuchungen zur physiologischen Morphologie der Tiere.*" II. Würzburg, 1891.